THE CRYSTAL STRUCTURE OF THE INOSITOLS AND THEIR COMPOUNDS.

Thesis presented to the University of Edinburgh
for the degree of Ph.D.

by

ANDREW MILLER.

May, 1962.

ABSTRACT OF THESIS

Name of Candidate ANDREW MILLER

Address [redacted]

Degree Ph.D. *Date* May, 1962.

Title of Thesis The Crystal Structure of the Inositols and their Compounds.

The crystal structure of myo-inositol dihydrate has been determined by an X-ray analysis.

The principal technique used in solving the structure was the Patterson method. This was first applied in projection when the additional help of a Partial Patterson synthesis was required to give a solution of two co-ordinates of the structure. A three-dimensional Patterson synthesis using 60% of the possible three-dimensional data (about 1,000 reflections) was used to solve the complete structure. Refinement was carried out first by Fourier methods followed up by the least-squares technique. The three-dimensional work was done on the D.E.U.C.E. Computer in Glasgow University.

The molecular structure was confirmed as being that suggested by Posternak i.e. the cyclohexane ring has one hydroxyl substituent in the axial position and the other five are equatorial. The C - C distances in the ring seem to be slightly shorter than the normal 1.54Å.

A maximum hydrogen bonding scheme obtains in the crystal structure in which the water molecules play an important part in holding the structure together, but an easy escape route for the water molecules is provided by channels in the structure and so the instability of the crystal in the atmosphere is explained.

Use other side if necessary.

TO

MY PARENTS

AND

ROSEMARY

C O N T E N T S.

1. INTRODUCTION.

The inositols - the hexa-hydroxy-cyclohexanes - comprise a highly important group of compounds. This is evidenced by the fact that the generally accepted criteria of importance are fully satisfied by the group as a whole. A considerable amount of work has been done and is being done on the chemical and biological aspects of these compounds but, to date, there has been only one series of papers - published in 1931 - on their crystal structure (1).

The first striking point about the inositols is their ubiquity. In one of Angyal's reviews (2) he says of myo-inositol, the most widely occuring of the group, 'No plant or animal tissue yet examined by an adequate method has been known to lack it. So it is probably present in nearly all living cells.' The inositols are also widely occuring in the form of the hexa-phosphate of myo-inositol which is responsible for 40% of the organic phosphate of the soil. When it is remembered that plants, animals and soil compose a considerable fraction of what we call 'Nature,' the significance of this group of compounds will be appreciated.

Wide-spread occurence is not the only criterion of importance satisfied by the inositols. To their ubiquity /

ubiquity is added a remarkable versatility of physiological and biological activity. They play important parts in the functioning of the major organs in animals such as the brain, nerves, heart, liver and kidneys, and they are thought to be of significance in some little understood pathological conditions like diabetes. They have vitamin effects in both plant and animal kingdoms and are of nutritional value in both. Bacterial activity is another sphere in which they play a role when they occur in streptomycin etc. Inositols may also form a link between the 'biological' and the 'organic' worlds by acting as intermediates in biosynthesis and in degradations of biological materials as well as being precursors for many other biologically occuring compounds.

Many other varied and interesting activities of the inositols could be listed and in fact, from a biological point of view, it seems impossible to exaggerate the importance of these compounds, but this importance does not leave the chemist untouched either. Barton and Cookson have shown in a review (3) the usefulness of the inositols in illustrating problems and methods in conformational analysis and the separation of the inositol isomers and the phosphate isomers have invoked some interesting physical methods.

Stereochemical Importance of Inositols.

As a series of compounds the inositols offer a unique opportunity for stereochemical study. This was appreciated by Patterson and White in 1931 (1) and its relevance is scarcely reduced today. It is clear from various considerations.

The inositols were the first series of compounds found to display optical activity and yet contain no assymetric carbon atom. This is, of course, a property of the cyclohexane ring itself, but also the inositols are the only set of hexa-substituted cyclohexanes of which every possible isomer is known, thus making feasible comparisons within a wide range of molecules of related structure. This is amply vindicated in one of Angyal's reviews where a large section is devoted to adducing examples of stereochemical problems illustrated by the inositol group.

In view, also, of the fundamental part played by myo-inositol in so many biochemical and physiological situations as well as its presence in all living cells, its vitamin activity and so on, and bearing in mind that the understanding of all these processes is obscure, it seems that some data on the simple inositol itself would lay a good stereochemical foundation for the biological theories. /

theories.

The stereochemical parallel of myo-inositol with glucose is also relevant. In going from one to the other the cyclohexane ring is replaced by a pyranose ring but the conformation of the possible hydroxy substituents is unchanged. A comparison of the C-O bond-lengths especially at the axial hydroxy group might shed some light on the short C-O bond problem referred to by McGeachin (4).

As long ago as 1951 R.A. Pasternack, after working out a related cyclohexene structure wrote 'A more accurate analysis of a simpler derivative of cyclohexane would be desirable' (5).

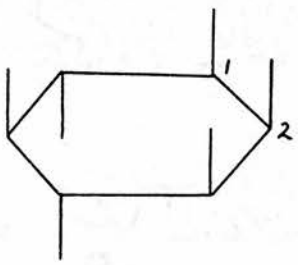
The Structure of myo-inositol.

About twenty years ago, practically nothing was known about the structure of the inositols, but in these last two decades chemical and conformational analysis have done much to clarify our understanding of these compounds (6), (7), (2).

The first steps in establishing the inositol structures are chemical. In the Scherer reaction the inositols are oxidised to hexa-hydroxy benzene thus showing their cyclohexanehexol structure. There are, however, nine possible stereoisomers of hexahydroxycyclohexane and in /

in the case of myo-inositol, proving its individual structure turned out to be difficult since oxidation with potassium permanganate, designed to degrade the ring, gave too many products from random fission. Progress was only made when stereospecific reactions were devised in 1942 by T. Påsternack (8) using enzymic dehydrogenation and Dangschat (9) who carried out chemical degradations to known carbohydrates.

These methods established that myo-inositol had the configuration given by

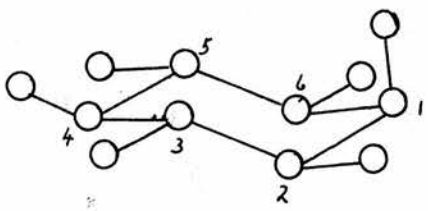


Applying to this the generally accepted tenets of conformational analysis viz.

(1) The most stable and so the most preponderant form of the cyclohexane ring is the chair form, and

(2) Substituents in the cyclohexane ring are more stable in the equatorial than the axial position, a conformation may be assigned to all the cyclitols and myo-inositol is given the one-axial five-equatorial conformation.

Numbering System Used



This is backed up by a number of chemical reactions of the cyclitols such as dehydrogenation and complex formation with borate. The latter type of reaction requires specific hydroxyl orientation and so may be used to give information about the relative orientations of the hydroxyl groups on the ring. As well as this, there is a body of physical evidence which lends cogency to the conformational arguments.

1. An absorption band at 873 ± 11 cms.-1 in Infra-Red spectra attributed to deformation of the C + H bond is found in all inositols except scyllo-inositol (all equatorial).
2. Nuclear Magnetic resonance spectroscopic studies which allow a distinction between hydrogen atoms in different positions, have been done on these compounds, and a comparison between the spectra of the cyclitols and the hexa-chloro-cyclohexanes has been made.
3. Dipole moments of cyclitol acetates give calculated agreement with expected conformations. (10).
4. Equilibrium constants of boric acid complexes give a method of determination of approximate free energy content of individual cyclitols. So it has been shown that myo-inositol having 1 axial -OH group and

5 /

5 equatorial is 9 k.cals./mole less stable than scyllo-inositol (all equatorial) (11).

A support of the above evidence is provided by the natural occurence of the inositols. All the isomers with two axial -OH groups on the same side of the molecule are unknown in nature, whereas all the other isomers are naturally occuring.

However, as recently as 1954, three French workers (12) after a discussion in the light of their own work and of statements by other workers (13), said that the 5 axial 1 equatorial conformation of myo-inositol could not be rejected. It is true that this statement was made before the publication of some of the more telling evidence listed above, but it does show that conclusive evidence of the existence of the 5 equatorial 1 axial conformation such as could be provided by X-ray crystal analysis, would be desirable.

The only publication on the X-ray investigation of crystals of the cyclitols is a series of papers by Patterson and White (1). There, space-group and unit-cell data is presented for a number of the cyclitols and in some cases tentative interpretations of the intensity data are put forward. In the case of myo-inositol dihydrate for example, a suggestion is made that evidence points to a tightly /

tightly bonded structure forming a comparatively open crystal structure. But, of course, no molecular information could be gained at this stage. In 1948 Bijvoet mentioned (14) that work was being started on myo-inositol and he wonders whether it will have the e a a a a a or a e e e e e conformation. Over a number of years this compound was studied by Bijvoet and Jellinck in Utrecht and their results (15) were kindly made available to us in this laboratory. No clear information on the molecular or crystal structure was advanced. Near the completion of the work described in this thesis we were informed by the 'Structures in progress' service of the Institute of Physics that Dr. Lomer of Birmingham University was also working on myo-inositol dihydrate, and a comparison was made of the final molecular and crystal structures derived in the two laboratories.

The Structure of Inositol phosphates.

The phosphates of myo-inositol, particularly the hexa-phosphate, are widely occuring in nature. No other inositol isomer except myo-inositol occurs in nature as a phosphate. The hexa-phosphate of myo-inositol, known as phytic acid, is, as was noted, responsible for 40% of the organic phosphate of the soil and also for a large proportion of the phosphate occuring in plants. Mono- and di- phosphates of myo-inositol have been isolated from brain /

brain and liver tissue.

Structurally very little is known of these phosphates. Examination of the mono-phosphate might be of use in confirming the theory that due to the slower hydrolising of the axial phosphate group, acid, alkali or enzymic hydrolysis of phytic acid all give the same 2-phosphate.

In the case of the di-phosphates, the 2, 4-isomer is known and it is optically active, so is indistinguishable chemically from the 2, 6-isomer. However, this isomer is readily available in the form of its barium salt and so X-ray examination using the anomalous dispersion method allowed by the presence of the barium atom, would settle the absolute configuration of the molecule.

Phytic acid itself has been the subject of a long chemical investigation by the aforementioned French workers (16). Their study was done by following neutralisation curves and conductivity changes in solution. Sodium phytate was neutralised with hydrochloric acid. The final results showed that phytic acid possessed four very feeble acid groups of $pK = 9{\cdot}7$, two feeble acid groups of $pK = 6{\cdot}3$ and six strong acid groups of $pK = 1{\cdot}8$. The authors then went on to scrutinise the various postulated structures for phytic acid in the light of their findings /

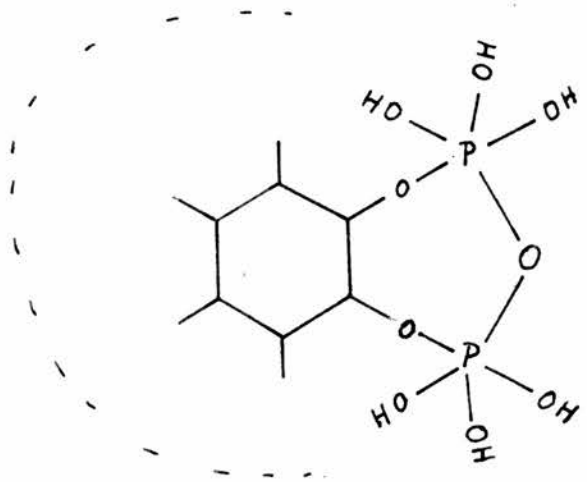
findings and to submit a suggestion for the spacial configuration themselves.

They reject Neuberg's formula (Fig. 1.a.), since it would possess 18 acid groups, and favour Anderson's, (Fig. 1.b), but not without restriction, because of their observed dissimilarity in pK values of the acid groups. They then go on to say that the evidence for a 1-axial 5-equatorial conformation for myo-inositol is not so certain as is generally implied, and hint that the 1-equatorial 5-axial form is just as probable or, at least, cannot be dismissed.

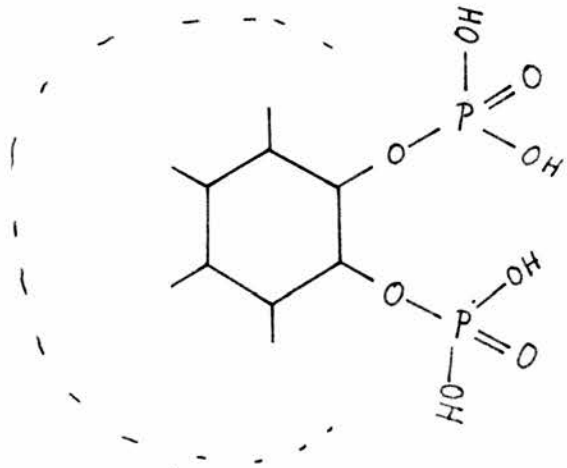
Proceeding from this, they suggest a structure for phytic acid involving the 5-axial 1-equatorial conformation, and explain that internal hydrogen-bonding between meta-substituted phosphate groups compensates for the extra energy introduced into the molecule by this conformation.

The formula suggested is (correcting an error in the formula in their paper) shown in Fig. 1.c. and it is remarked that this formula has the advantage of perfectly explaining their results. While this structure does fit their results, it must still be placed in the realm of speculation when it is remembered that in the solid state each molecule of phytic acid is associated with 36 molecules of water which are able to take part in a hydrogen-bonding scheme; so again the point emerges that the type of information than an X-ray analysis affords would be admirably suited to settling how correct are the chemists' interpretation of their own results.

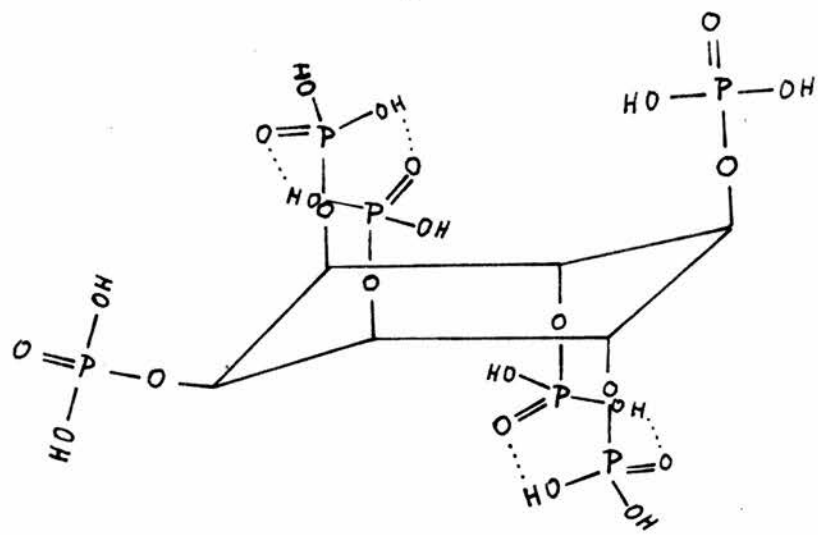
Fig. 1.



1.a.



1.b.

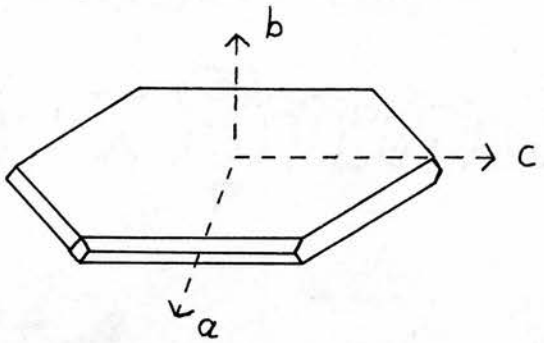


1.c.

2. Determination of Structure of myo-inositol dihydrate.

(a) Space Group and Unit Cell Dimensions.

Crystalline myo-inositol may be obtained either in the anhydrous form or as a dihydrate. The former is poorly crystalline and can be crystallised only with difficulty from a mixture of water and acetic acid or as Lomer suggests (17), from water above 50°C. The dihydrate, on the other hand, crystallised readily from distilled water giving crystals up to 1cm. long, 0.5 cms. broad and 0·1 cm. thick. On examination under the microscope they were seen to have the shape



The axes shown are in the directions of what later proved to be the crystallographic axes.

A razor blade was used to cut the crystals to the shape of cylinders about 1 mm. long and 0·2 mm. in diameter, the length in each case, being along one of the three axes. A cleavage plane was noted perpendicular to the shown b-axis. The crystals, while stable in the mother /

mother liquor, were found to decompose into a powder in the atmosphere, this decomposition being complete after about 12 hours exposure. X-ray powder photographs were taken of these decomposed crystals and they were identical with powder photographs obtained on ground crystals newly removed from the mother liquor. So if this decomposition involves the loss of water by the dihydrate crystal, it seems that it also loses water on grinding.

For the purpose of doing X-ray work on the dihydrate, various attempts were made to prevent or retard this decomposition of the crystal in the atmosphere. The crystals were dipped in collodion dissolved in ether, the ether then being allowed to evaporate off, leaving a thin film of collodion on the surface of the crystal. This procedure was repeated several times to thicken the collodion coat and it was found to delay decomposition for about 1 day. Later, it was discovered that coating the crystal surface with a thin film of Vaseline delayed decomposition for up to 1 week, and this had the advantage that Vaseline could also be used as an adhesive, keeping the crystal on the glass rod for mounting in the X-ray beam. On long exposure, heat tended to soften the Vaseline and the crystal moved, so during the long term photography for 3-D data, Vaseline was used as a coating and seccotine as an adhesive.

The /

The X-ray source was a Metropolitan Vickers Raymax unit running at 20 m.A. and 50 K.V. using a copper target. Copper $K\beta$ radiation was filtered off with nickel foil. The films used in photography, Ilford Industrial G and Industrial B, were developed for 7 mins. in Ilford Phen-X developer. When intensities were to be measured, the triple-film overlay method was used. Two Industrial B films were placed behind an Industrial G in a 5 cms. radius, vertical travel Weissenberg camera (Beevers 1952 (18)). The ratio of the intensities of identical spots between one another on the three films G: B_1: B_2 was $7\frac{3}{4}:2\frac{1}{2}:1$.

Oscillation and Weissenberg photographs about the three axes gave information on the space-group and unit cell dimensions. It was clear from the symmetry of the Oscillation photographs that the crystal was in the monoclinic system, so the unique axis was taken as the b-axis, and the smaller of the remaining two as the C-axis in accordance with international convention.

Systematic absences were observed on the Weissenberg photographs as follows:-

1.	a-axis	(o k o) when k = odd.	Suggests a screw-axis parallel to b-axis.
2.	b-axis	(h o l) when h = odd.	Suggests a glide-plane perpendicular to b-axis with glide parallel to a-axis.

3. /

3.	c-axis	(o k o) when k = odd.	Suggests screw-axis parallel to b-axis.
		(h o o) when h = odd.	Special case of 2.

So the crystal, as White pointed out (1), is in space-group C_{2h}^5, and using the above system for naming the axes, this is $P2_1/a$ - perhaps the most common space-group of organic crystals. The presence of a centre of symmetry was confirmed by a statistical test on the intensities (19) and by the absence of piezo-electricity in the crystal as tested on a Geibe-Schiebe instrument.

Approximate values for the reciprocal lattice unit cell dimensions were determined from the positions of reflections of the type (h o o). To obtain an accurate value for b^*, spots near the shadow of the knife-edges were selected with high k index and low l index. The distance from the knife edge shadow was converted to the Bragg angle θ by means of a calibration graph obtained by measurements done on a quartz powder specimen. Values of b^* thus obtained were plotted against $\sin^2\theta$ and extrapolation to $\sin^2\theta = 1$ gave a value of b^* free from error due to film shrinkage. In a similar way accurate values were determined for a^* and c^*. In the case of β^*, the plot of β^* against $\sin^2\theta$ was not a straight line, so the mean value /

value of the results was taken.

The dimensions found were

$a^* = 0.18146$ $\alpha^* = 90^\circ$

$b^* = 0.09162$ $\beta^* = 68^\circ 11'$

$c^* = 0.25409$ $\gamma^* = 90^\circ$

Hence, using $\lambda_{Cu K\alpha_1} = 1.5405$ Å

$a = 9.144 \pm 0.005$ Å $\alpha = 90^\circ$

$b = 16.814 \pm 0.005$ Å $\beta = 111^\circ 49' \pm 5'$

$c = 6.531 \pm 0.004$ Å $\gamma = 90^\circ$

Density measurements carried out by the floating method in a mixture of ethyl alcohol and bromoform gave $\rho = 1.54$ gms./cc. This indicates 4 molecules $C_6H_{12}O_6.2H_2O$ in the unit cell.

(b) Determination of Approximate Structure (2 - co-ordinates)

The proposed method of solving the structure was the Patterson method. This finds its widest application when the crystal contains a heavy atom, because the vectors due to the heavy atom are readily identified and once the heavy atoms have been placed, superposition or Fourier methods can be used to find the light atoms. However, the Patterson method is also a convenient way of getting some idea of the crystal structure when the molecular structure is roughly known. In this event intra-molecular vectors can be predicted on the basis of the molecule, and then sought in the Patterson. Full use can be made of any symmetry elements in the space-group by predicting inter-molecular vectors and then searching for them in the Patterson. This is a specially powerful method when the molecule contains parallel vectors because these can easily be identified in the Patterson and so their orientation in the structure is known. In the myo-inositol molecule

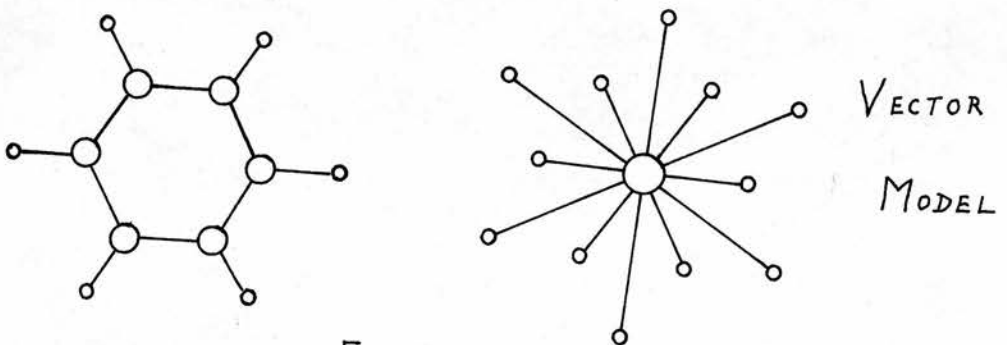


FIG. (a).

there are many parallel vectors and, in fact, a predicted vector /

vector model based on the inositol molecule can be envisaged (Fig. (a). c.f. Macdonald (20) Beevers and Ehrlich (21)). The vector model can then be rotated around the origin of the Patterson until a fit is obtained and so the molecular orientation is indicated (possibly with accompanying ambiguity). After that, the Patterson may be inspected for evidence of vectors between two molecules related by a centre of symmetry, or for vectors of the Harker type which will fix the position of the molecule in the unit cell. This was the general plan for attack on the inositol structure.

Two-dimensional intensities were collected by means of triple-film Weissenberg photographs taken about three axes. Visual estimation gave these intensities to an accuracy of about 10%. Since any correction for absorption of X-rays by the crystal would be of a smaller order than this accuracy, no such correction was applied. The intensities were converted to $F^2_{rel.}$ by application of the Lorentz and polarisation factors and then scaled by Wilson's statistical method to give $F^2_{abs.}$ Using these values, the Patterson functions given by

$$P(u\ v\ w) = \frac{1}{V} \sum_h \sum_k \sum_l {}^{+\infty}_{-\infty} |F(h\ k\ l)|^2 \exp 2\pi i\ (hu + kv + lw)$$

were calculated for the three projections.

The F(h k o) and F(o k l) reflections were then 'sharpend ' /

'sharpened'. The normal type of sharpening functions used are equivalent to inverse temperature effects (see Buerger p. 64 when it seems $\exp\frac{-B\sin\theta}{\lambda}$ should be $\exp\frac{-B\sin^2\theta}{\lambda^2}$) but in this case for empirical reasons based on previous experience in this laboratory, the function used was exp (2·6 sin θ). The effect of this function is to make the peaks of Patterson more clearly defined so that recognising peak positions may be an easier task. However, increasing the value of the F^2 of the higher order reflections is tantamount to increasing series-termination effects since the amplitudes of the Fourier terms have still a considerable magnitude when they are cut off by the 'reciprocal sphere limitation.' In the Fourier map these series-termination effects show themselves as diffraction ripples (circles of negative ground) round the true Fourier peaks, and it is in this respect that 'sharpening' may be dangerous because the diffraction ripples round some large peak may swamp a neighbouring smaller peak. 'Sharpening' will be of greatest use in two-dimensional Fourier 'projections' since there peak-recognition is going to be most arduous, and yet it is also in projection that the diffraction ripples are likely to prove most malicious because there is no limit to how close peaks may be in projection. For this reason it is expedient to calculate an unsharpened Patterson first so that /

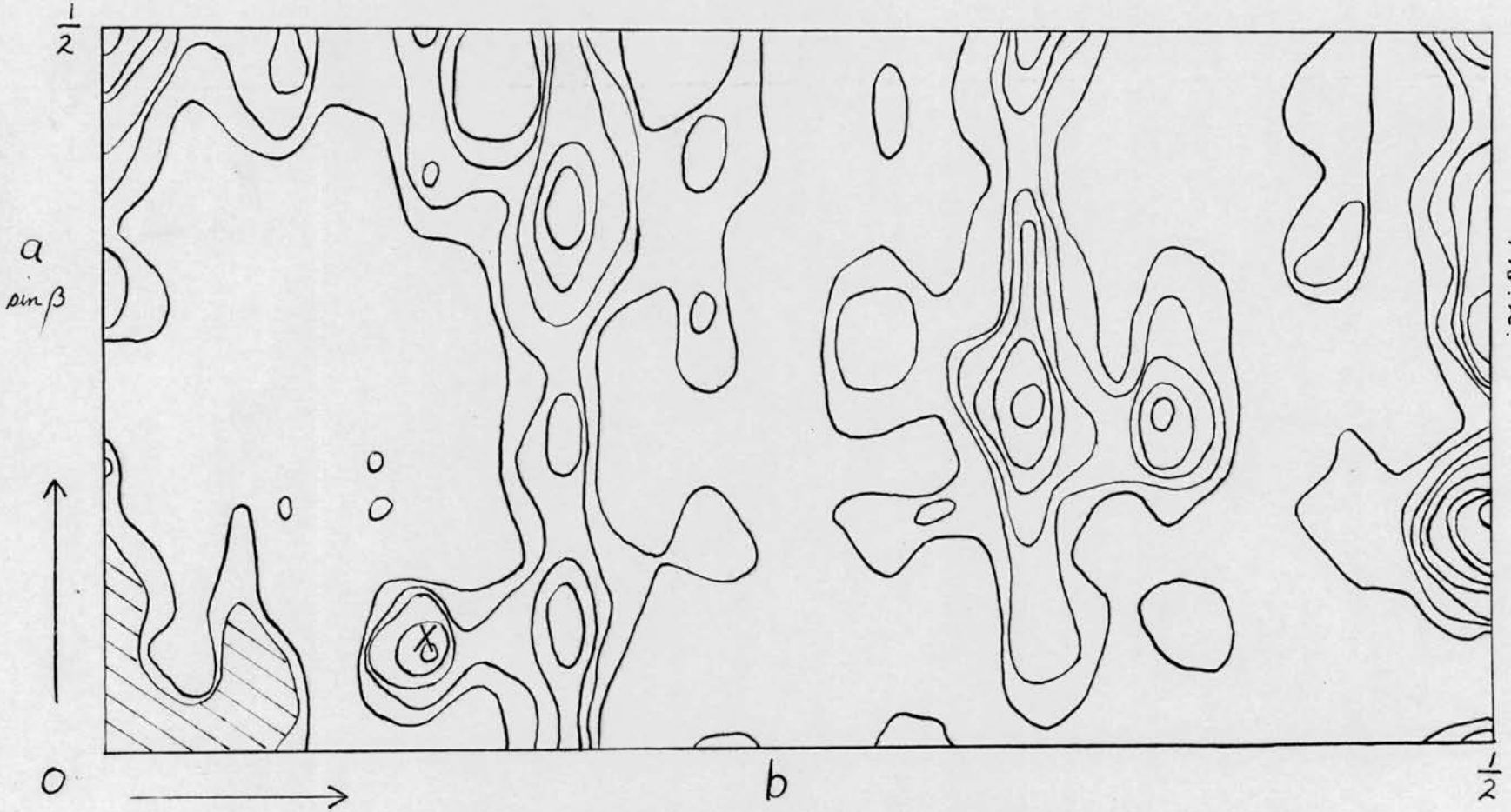


FIG. 2.

C-AXIS PATTERSON PROJECTION

that the effects of sharpening may be clear.

In the present type of problem it seems that sharpening provides a most useful function in high-lighting the Patterson peaks. It is true that with crystals containing a heavy atom, sharpening is not normally needed to find the heavy atom positions, nor is it of any assistance if some analytical type of image-seeking function is used to locate the lighter atoms; but in the case of a light atom structure where the methods referred to above are being employed in an attempt to solve the structure, in short, when the Patterson map is being regarded and used as a point vector set of the true structure, then 'sharpening', if carefully applied, may prove useful in defining more clearly the peak positions. In the case of myo-inositol, 'sharpening' helped considerably, especially in the (0 k 1) projection, in showing up the vectors.

The c-axis 'sharpened' Patterson is shown in Fig. 2. At this stage the map did little more than to emphasise by its striations the dominant 6th, 12th and 18th order (0k0) reflections. The shape of the origin peak gave some indication of a short vector and this along with a peak at about 2°_{A} from the origin (labelled X) suggested an orientation of the ring perpendicular to the a - b plane and at an angle of about 20° to b -axis.

Harker /

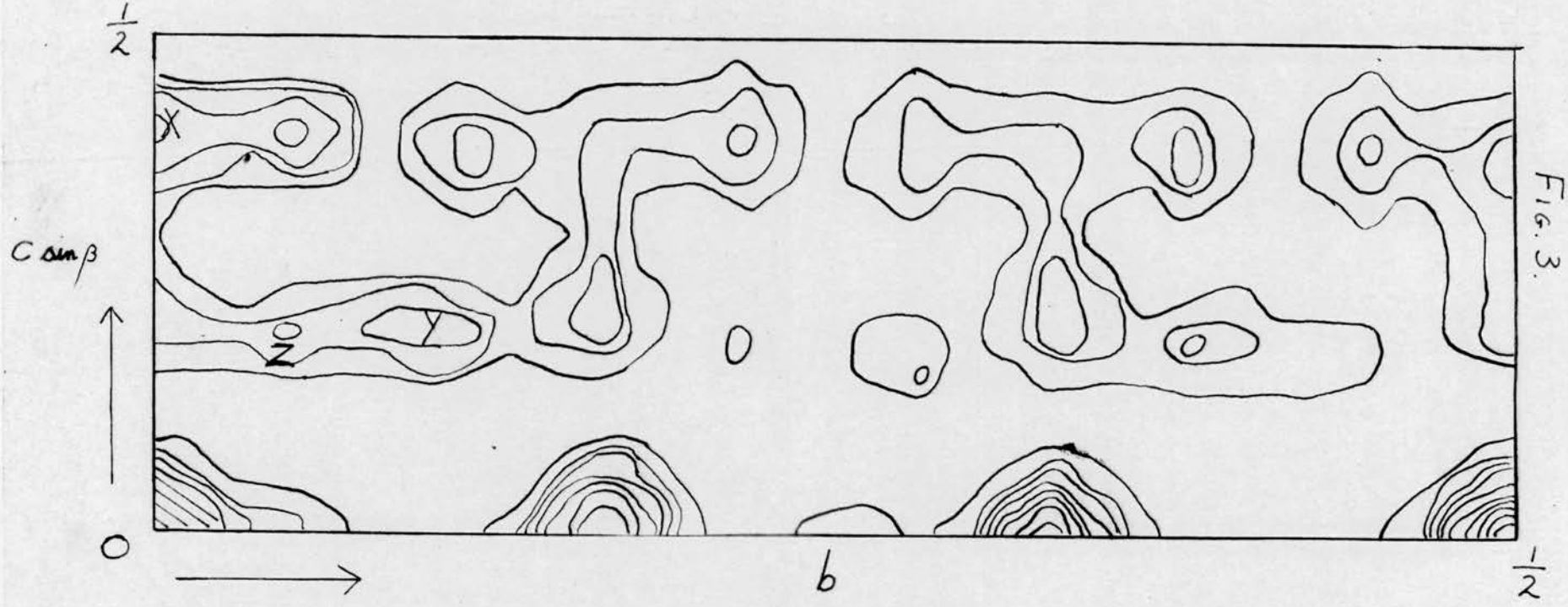


FIG. 3.

α - AXIS PATTERSON PROJECTION

Harker peaks are clear at $y = \frac{1}{2}$ (screw axis) and $x = \frac{1}{2}$ (glide plane) but their interpretation is not so clear. However the striations of peaks are about 2·7Å apart along the b-axis and this is about the same length as the projection of the parallel vectors between atoms $C_1 - C_4$, $O_2 - O_3$ and $O_5 - O_6$ on the b-axis. (See page 5 for numbering).

Fig. 3 shows the a-axis Patterson. The following features are evident from this map.

(i) There are lines of peaks parallel to b-axis confined to $z = 0$, $z = 1/5$ and $z = 2/5$.

(ii) There are two peaks at 2·5Å from origin (labelled X) and four peaks at 2·1Å from origin (labelled Y).

(iii) There are four peaks around the origin at a distance of 1·5Å (labelled Z).

(iv) A heavy Harker peak occurs at ($y = \frac{1}{2}$, $z = 0$) and two heavy peaks at ($y = 1/6$, $z = 0$) and ($y = 1/3$, $z = 0$).

The first point suggested by these facts is that the molecule is situated nearly symmetrically on the b-axis. This is clear because at least one of the large peaks at $y = 1/6$ and $y = 1/3$ will be due to the vector between two molecules related by a centre of symmetry, and it is at $z = 0$. Again the Harker peak due to the screw axis parallel to the b-axis, is at $z = 0$ indicating that the molecule is on the b-axis. A third observation is that the line $y = \frac{1}{2}$ is nearly (but not exactly) a minor plane in the Patterson. Now if the molecule (and its accompanying two water molecules) were /

were perfectly symmetrically disposed about the b-axis, the (o k 1) reflections would be absent when k = odd, and the Patterson map would have a mirror plane at $y = \frac{1}{4}$. Since this feature is closely approximated in the Patterson it indicates that any deviation from the symmetrical disposition is not large.

Following on from this, the map suggests that the inositol molecule is orientated with the $C_1 - C_4$ vector nearly parallel to the b-axis, because it is this orientation which allows the axial hydroxyl group to be nearly on the b-axis. If the axial hydroxyl group was far removed from the b-axis this would introduce a greater disymmetry about the b-axis than the weak k = odd reflections seem to indicate. This is supported by the fact that there are no peaks between the rows at z = 0, z = $^1/5$ and z = $^2/5$. In projection the distance corresponding to z = 1/5 is $\frac{c \cos\beta}{5}$ i.e. about $1{\cdot}2^{\circ}_{A}$. Now this is half the length of the vector between $C_2 - C_6$ ($2{\cdot}5^{\circ}_{A}$) and $\frac{2C \cos\beta}{5}$ is about half the length of the vector between $O_2 - O_6$. So the indication is that these vectors $C_2 - C_6$, $O_2 - O_6$ are perpendicular to the b-axis and parallel to the plane of a-axis projection (i.e. perpendicular to the a-axis).

It only remains now to determine the angle between the $C_1 - C_4$ vector and the b-axis and the molecular orientation /

orientation will be fixed. The $C_1 - C_4$ vector is parallel to the $O_2 - O_3$ and $O_6 - O_5$ vectors and is of length 3°_{A}, so it is expected to appear as quite a heavy peak in the Patterson somewhere along the b-axis and within 3°_{A} of origin. The large peak at $y = {}^{1}/_{6}$ (i.e. $2{\cdot}6^{\circ}_{A}$ from origin) satisfies these conditions and gives an angle of about 54.5° between the $C_1 - C_4$ vector and the b-axis. This angle is half the tetrahedral angle of $109^{\circ}28'$. In such an orientation the peaks at $1{\cdot}5^{\circ}_{A}$ and $2{\cdot}0^{\circ}_{A}$ from the origin are now well accounted for in terms of the cyclohexane ring $1{\cdot}54^{\circ}_{A}$ and $2{\cdot}5^{\circ}_{A}$ vectors - one of the shorter vectors occurring as a deformation of the shape of the origin peak along the b-axis.

It would seem probable that the large peak at ($y = {}^{1}/_{3}$, $z = 0$) is due to the vectors between the two molecules related by a centre of symmetry. This peak is large because the molecule itself is nearly perfectly centrosymmetrical. The axial hydroxyl group and possibly the positions of the water molecules detract from a perfect centre of symmetry, but the rest of the molecule has a centre. The large peak at ($y = {}^{1}/_{6}$, $z = 0$) would appear to give too short a vector for that between two centrosymmetrically related molecules of inositol.

Reverting now to the c-axis Patterson projection, it is seen that the orientation and position of the molecule explains /

explains many of the features of this map. The striations of peaks at y = $^1/_6$ and $^1/_3$ are due to $C_1 - C_4$ and parallel vectors. The shape of the origin is accounted for by the short (1·5Å) vectors of the ring and the peak at 2Å from the origin by the 2·5Å ring vectors. It may be noted too that the high ground at y = $^1/_3$ is concentrated into one large peak and there are also peaks at 1Å and 2Å from it, so this certainly appears to be the centre-centre peak. A closer analysis of this map was made later when the x-co-ordinates of the atoms were being determined, but here it might just be noted that the map may be regarded as equally spaced striations of peaks parallel to the a-axis and separated by a distance of $\frac{b}{18}$ i.e. about 0·93Å which is nearly the same as the projection of the C - C vectors (1·54Å) on the b-axis.

It was at this stage, and after the preceding reasoning, that the essential features of the whole structure were realised. The rest of the work proved to be a lengthy improvement and refinement of the atomic positions. The a-axis projection of the structure as it was now conjectured is shown in Fig. 4.

The molecules 1 and 5 are related to each other by a centre of symmetry at (y = $\frac{1}{2}$, z = 0). Molecules 3 and 4 are related to molecules 1 and 2 by the a-glide plane and so have x-co-ordinates equal to the x-co-ordinates of the molecules /

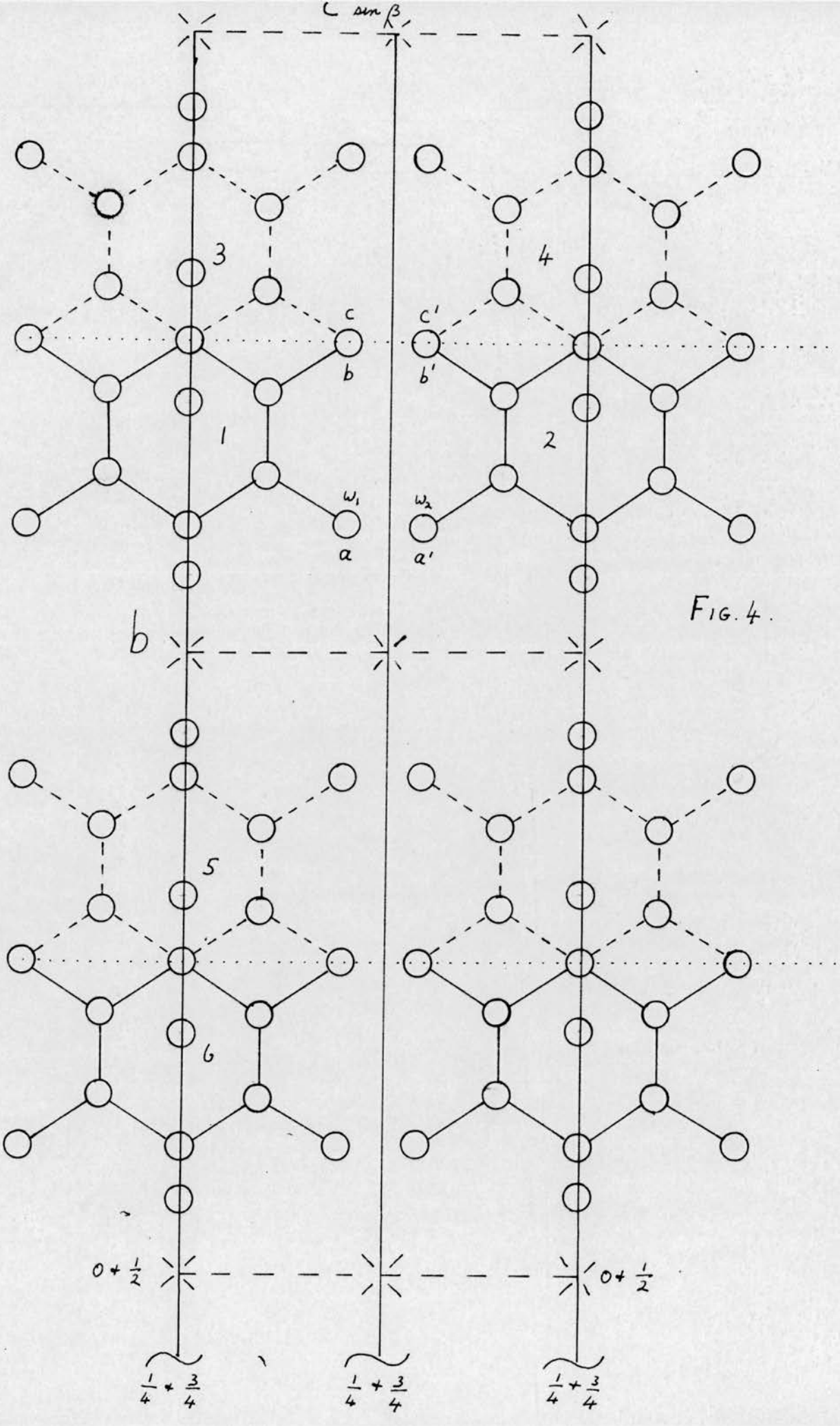


FIG. 4.

molecules 1 and 2 increased by $\frac{1}{2}$. Molecule 6 is related to molecule 1 by the screw axis at x = $\frac{1}{4}$ parallel to b-axis. Now the height of molecule 2 above molecule 1 in the a-axis direction is given by c sin (β - 90°) i.e. 2·42$\mathring{A}$ and this is nearly the same as $\frac{a}{4}$ = 2·29$\mathring{A}$. It follows then, that in going through the sequence of molecules 1, 2, 3, 4 nearly equal steps in the a-axis direction are involved, viz. $\frac{a}{4}$. With the molecules in the above orientation the distance between hydroxyl group a of molecule 1 and hydroxyl group a^1 of molecule 2 was calculated as being from 2·5 - 3·0$\mathring{A}$. The distances b - b^1, (molecules 1 and 2), b^1 - c (molecules 2 and 3) and c - c^1 (molecules 3 and 4) are also of this order. It was therefore postulated that the molecules 1 and 2, 2 and 3, 3 and 4, were held together by a zig-zag chain of hydrogen bonds running in the sequence b b^1 c $c^1 b_1$ where b_1 is b in next unit cell along the a-axis. Hydroxyl groups a and a^1 were also taken to be joined by a hydrogen bond and the positions of the two water molecules was suggested as follows. Water molecule w_1 was said to have the same y and z co-ordinates as a but its x-co-ordinate increased by $\frac{1}{2}$, similarly water molecule w_2 had same y and z co-ordinate as a^1 with its x-co-ordinate also increased by $\frac{1}{2}$. So now a zig-zag chain of hydrogen bonds may be imagined as running in the sequence a $a^1 w_1 w_2 a_1$ where a_1 is a in the next unit cell along the a-axis.

Futhermore /

Furthermore it was speculated that since water molecules usually hydrogen bond tetrahedrally, w_1 would probably be bound to the nearest b-axis hydroxyl groups of molecules 3 and 5, and so the x-co-ordinates of these hydroxyl groups would be nearly the same and nearly equal to that of w_1. So the relative positions of molecules 3 and 5 in a-axis direction are established, hence relative positions of molecules 1 and 5 and so the distance of molecule 1 along a-axis from the centre of symmetry. This structure accords well with all the evidence thus far at hand. The water molecules are symmetrically placed round the b-axis as the weak k = odd (o k l) reflections indicated.

Structure-factors were calculated for an (o k l) trial structure. At this stage the water molecules were omitted from the calculations because no direct evidence as to their positions had, as yet, come from the intensities. A comparison between F obs. and F calc. showed a temperature factor of exp $(-1{\cdot}19\frac{\sin^2\theta}{\lambda^2})$, so this was applied to the F calc.^{s}. A Difference map, i.e. a Fourier using (Fo - Fc) as Fourier co-efficients (where Fo's were given signs indicated by the corresponding Fc's) was calculated to see if any indication of the positions of the water molecules and/or the nature of the divergence of the molecule from perfect symmetry about the b-axis. Little success was encountered, the Difference maps being, on the whole, featureless.

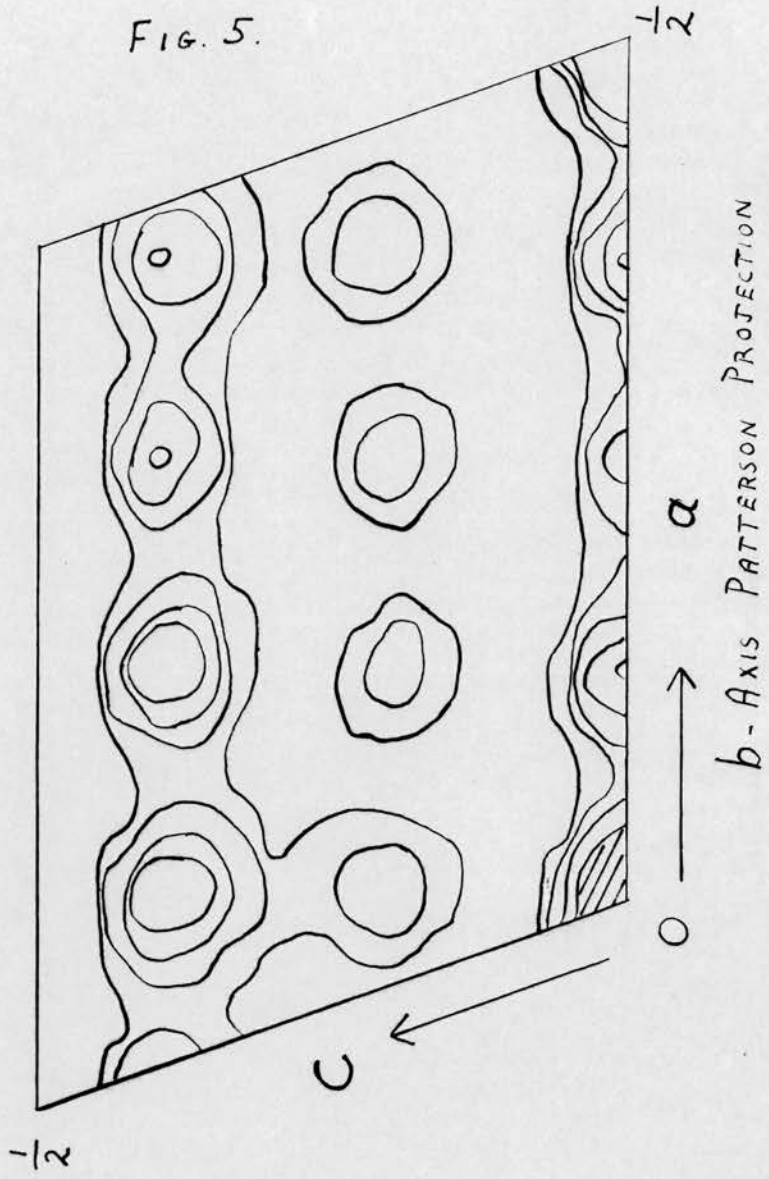
featureless.

The b-axis Patterson was now calculated. Even without an applied 'sharpening' function (see Fig. 5) it showed the same neat, well resolved rows of peaks at z = $^1/_5$ and $^2/_5$ as were observed in the a-axis Patterson and it confirmed that the orientation of the molecule is such that the C_2 - C_6 and parallel vectors are at right angles to the a-axis. Unfortunately in this long axis projection there is a fair degree of overlapping and it did not prove possible to recognise from it either the positions of the water molecules or the position of the molecule itself up the a-axis.

The Harker peak at x = $\frac{1}{2}$ in this Patterson, since it is due to the glide-plane does not provide much useful information about the position or orientation of the inositol molecule. A hint about the presence of the water molecules is given in that the peaks in the row at z = $^2/_5$ are not all of the same weight. This certainly seems to support the idea that the water molecules will have a z-co-ordinate which is equal to that of other hydroxyl groups of the molecule i.e. about z = $^2/_5$, and so they may well be placed so that they can participate in the hydrogen bonding of a type conjectured on pages 24 and 25.

Rows of peaks may also be noted in this b-axis Patterson /

FIG. 5.



b-AXIS PATTERSON PROJECTION

Patterson which are perpendicular to the a-axis. As stated above, this confirms that the vectors $C_2 - C_6$ and those parallel to it, are perpendicular to the a-axis. But since these rows are separated by a distance of one eighth of the a-axis i.e. $1{\cdot}1^{\circ}_{A}$, it confirms the tilt on the molecule which raises the $C_3 - C_5$ vector about $1{\cdot}2^{\circ}_{A}$ in the a-axis above the $C_2 - C_6$ vector. According to the structure suggested so far, this distance should in fact be about $1{\cdot}25^{\circ}_{A}$ i.e. $1{\cdot}54 \sin (\beta - 90^{\circ})^{\circ}_{A}$.

It may be noted that in this Patterson map the $(\bar{1},4)$ line is nearly a mirror-line. This corresponds to the fact that in the Weissenberg photograph, the $(\bar{1},4)$ line is also nearly a mirror-line. If the inositol molecule had been centrosymmetric in this projection then the $(\bar{1},4)$ line (because it defines the orientation of the molecule in this projection) would have been a perfect mirror-line both in the photograph and the Patterson. The fact that the hydroxyl group on C_1 is axial and not equatorial is probably mainly responsible for the deviation from perfect symmetry. An accompanying disymmetrical effect from the water molecules could be envisaged, but since the overall effect is not large, and since the axial hydroxyl group effect is there already, it would seem that the water molecules are probably nearly centrosymmetrically situated in this projection. The $(\bar{1},4)$ diagonal also proved to have a spacing nearly equal to that of the a-axis - a fact which demanded some care in indexing the /

the Weissenberg photographs.

Some careful consideration was now given to the weak k = odd (o k l) reflections. If the whole molecule, including the water molecules, were symmetrically disposed about the b-axis, then the k= odd (o k l)reflections would be zero. As it is, $\sum F^2(okl)k=odd$ is leas than one tenth of $\sum F^2(okl)k=even$, so these weak k = odd intensities should contain all the information about the deviation from perfect symmetry about the b-axis. Since attempts to find the nature of this deviation by means of (Fo - Fc) map had failed, it was felt that the reason was that since on the previously postulated symmetrical structure no Fokl with k= odd were calculated, so no signs could be given to the Fokl with k = odd, the Fo - Fc) map which excluded the Fokl k = odd contributions would just not contain enough of this type of information to show up. It is not true to say that the (Fo - Fc) map using only Fokl k = even reflections contains no information on the deviation from symmetry, because the k = even reflections will have different calculated values in the disymmetric orientation than in the symmetrical case. But this information will be a second-order effect independent of the sign of the change and would probably be swamped by the general inaccuracy of the structure plus the large (Fo - Fc) contributions resulting from the exclusion of the water molecules from the Fc calculations.

It /

It was therefore decided to try to obtain from the k = odd reflections all the information they could yield as this should be entirely about how the structure deviated from the symmetrical orientation. The additive property of Fourier series was the basis of the method used. A glance at the a-axis Patterson (Fig. 3) shows that it nearly has a mirror plane parallel to the z-axis at $y = \frac{1}{4}$. Now the sum of the contributions from the F^2okl (k=even) and F^2okl (k=odd) reflections determines the form of the complete Patterson, but it is the contribution from the F^2okl (k=odd) reflections alone that determines the deviation from the true mirror symmetry about the line $y = \frac{1}{4}$. A Patterson map using the F^2okl (k=even) reflections only would have a true mirror plane at $y = \frac{1}{4}$. So a Partial Patterson using the F^2okl (k=odd) reflections only, given by

$$\partial P(v,w) = \frac{1}{A} \sum_{l} \sum_{k=2n+1} |F_{okl}|^2 \sin ky \sin lz$$

was calculated.

In Fig. 6. this map is shown. The negative ground is dotted and is uncontoured. An interesting fact about its symmetry may be noted. It has a reversal mirror plane at $y = \frac{1}{4}$. This means that any peak at (y,z) has a corresponding identical negative 'peak' at $(\frac{1}{2} - y, z)$. This is the sort of symmetry discussed by Cochran (22). He observes that although this type of symmetry is not normally encountered by crystallographs because they are usually dealing /

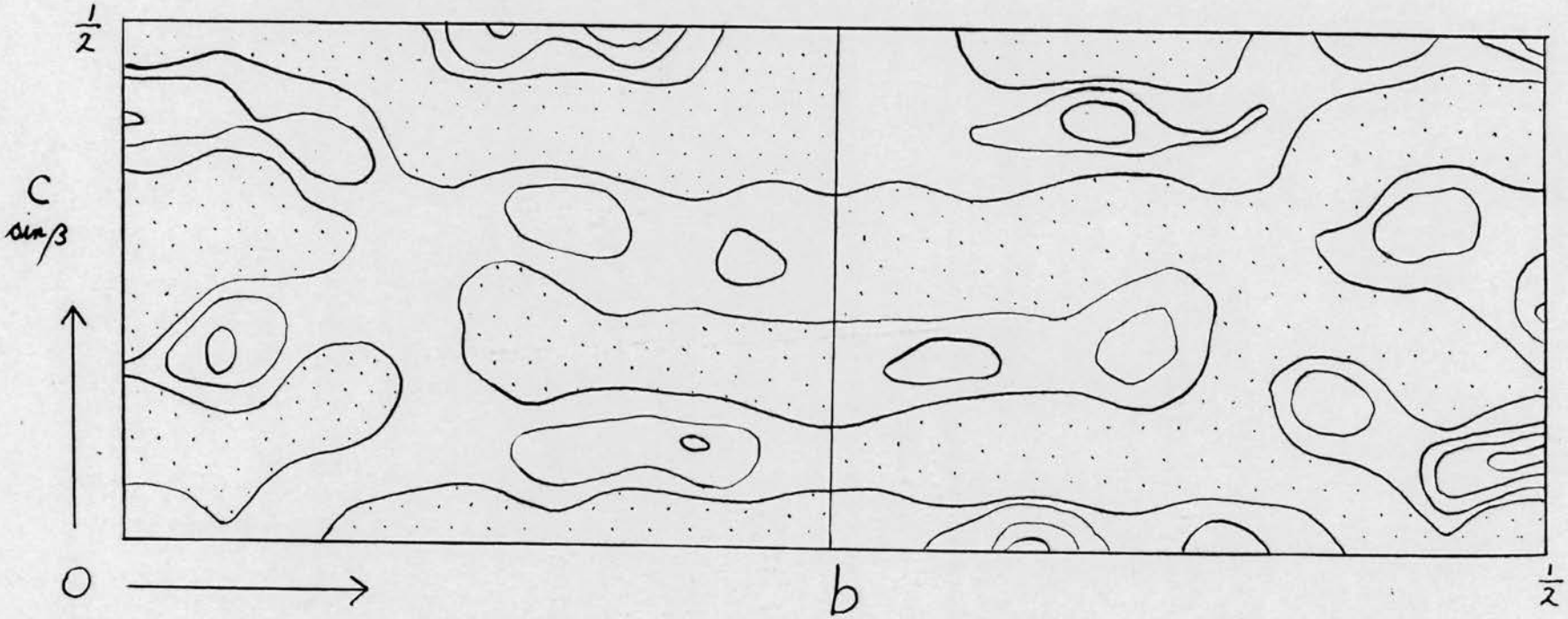


FIG. 6.

PARTIAL PATTERSON — USING (0kℓ)s WHERE k = ODD ONLY

dealing with distributions of factors like electron density which are always positive, yet it does occur when generalised projections are dealt with. A list of 46 reversal space groups in two dimensions is given in Cochran's paper and it appears that in our case, the Partial Patterson is in the group pm + m⁻. This map could also have been obtained directly from the complete Patterson in the following manner. Let p_1 represent the height in the full Patterson of any point (y,z) and p_2 the height of any corresponding point $(\frac{1}{2} - y,z)$. For each obtained p_1, form $\frac{p_2 - p_1}{2}$ and enter this with a negative sign at the site of p_1, and with a positive sign at the site of p_2. This clearly preserves the reversal mirror plane m⁻ at $y = \frac{1}{4}$. The line $y = \frac{1}{4}$ is actually the zero contour, and on it we have the unusual feature of contour lines meeting. So the drawing of the Partial Patterson is just a method of high-lighting the points in the full Patterson where the $y = \frac{1}{4}$ mirror does not apply, and so of clarifying the nature of deviation of the structure from perfect symmetry about the b-axis.

In the interpretation of the Partial Patterson, it was assumed that the water molecules are not the sole cause of the weak k = odd reflections. This is a reasonable assumption because evidence so far obtained indicates that the water molecules are probably situated nearly symmetrically about the b-axis at $z = \pm 2/5$. Concentrating our attention /

attention on the inositol molecule, there are three principal ways in which deviation from perfect symmetry about the b-axis may be achieved:-

(i) The centre of the molecule could remain on the b-axis with the rest of the molecule moved slightly off the axis by a small rotation (of the order of three or four degrees) about its centre.

(ii) The whole molecule, including its centre, could be moved slightly off the axis in a direction at right angles to the axis, but no rotation applied.

(iii) A combination of effects (i) and (ii) in which the centre of the molecule is off the axis and a small rotation of the whole molecule about its centre is applied.

We can now use the Partial Patterson to decide which of these possibilities is the one which exists, because the effect that each possibility will have on the Paftial Patterson can be predicted. This is illustrated in Fig. 7.

In case (i) the peak arising from the vector between two centrosymmetrically related molecules will remain on the b-axis because the molecular centres are still on the b-axis. However, the Harker peak due to the vector between two molecules related by the screw-axis parallel to the b-axis will be slightly off the b-axis.

In case (ii) the centre to centre peak will be off the b-axis and so will the Harker peak. A similar situation will arise in case (iii) so it will be difficult to distinguish between the second two possibilities.

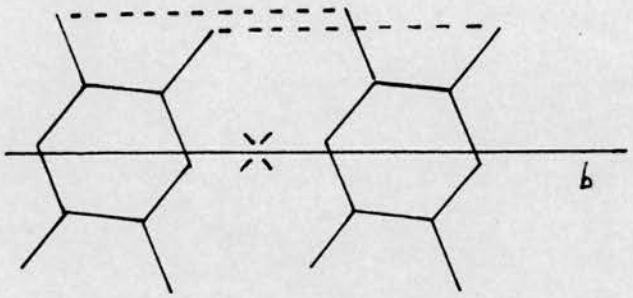
Examination /

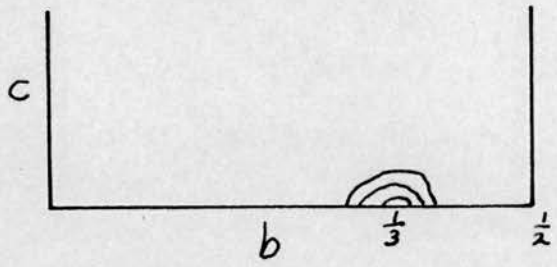
FIG. 7.

PARTIAL PATTERSON PEAK EXPECTED

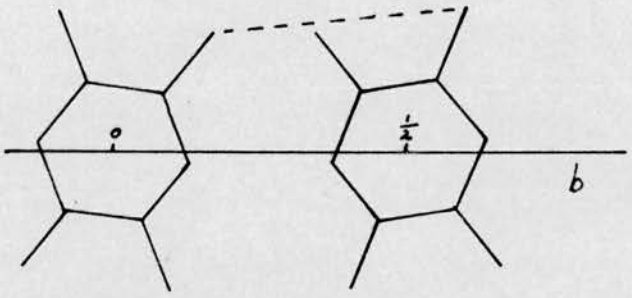
CASE (i)

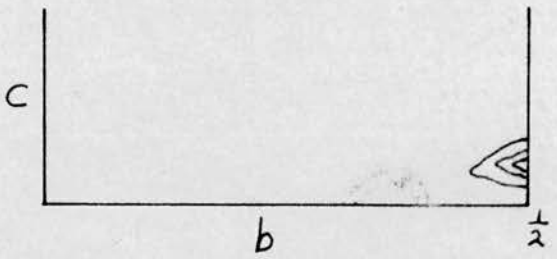
CENTRE - CENTRE PEAK





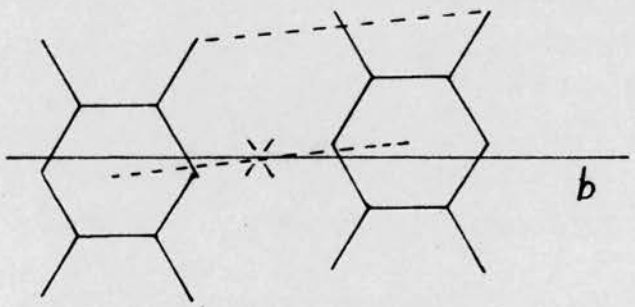
HARKER PEAK

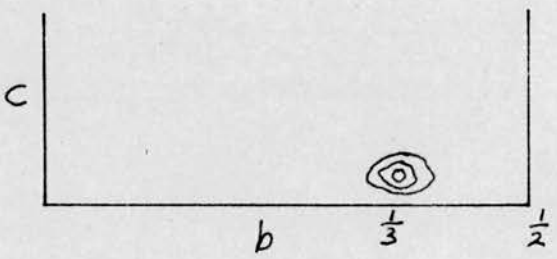




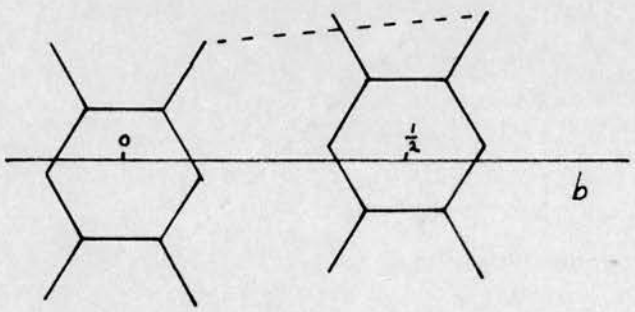
CASE (ii)

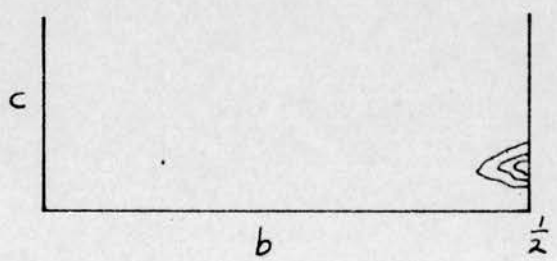
CENTRE - CENTRE PEAK





HARKER PEAK

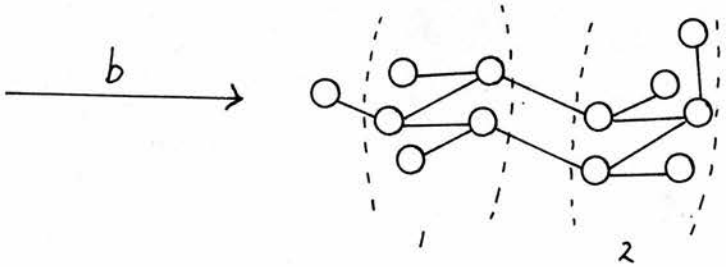




Examination of the Partial Patterson shows that the centre to centre peak at ($y = {}^{1}/3$, $z = 0$) in the full Patterson, is still on the b-axis in the Partial Patterson, while the Harker peak at $y = \frac{1}{2}$ is moved off the b-axis in the Partial Patterson. This clearly indicates that the deviation from perfect symmetry about the b-axis takes the form of a small rotation of the molecule about its centre - the centre remaining on the b-axis.

A final point may be mentioned on the interpretation of the Partial Patterson. It is not, by itself, to be regarded as a vector map. It merely shows the way in which the deviation from symmetry about the b-axis changes the vector map (i.e. the full Patterson). Thus the existence of negative areas in the map is explained. The point behind the interpretation is that the particular deviation from b-axis symmetry in the case of inositol does not change (shift) the centre-centre peak off the b-axis, whereas it does move the Harker peak.

It may be noted that the peak in the full Patterson at ($y = {}^{1}/6$, $z = 0$) is not moved off the b-axis by the Partial Patterson. The explanation for this involves the fact that the inositol molecule may be regarded as composed of two sections along the b-axis.



The /

The peak at $y = 1/6$ is due to intermolecular vectors between sections 2 and 1 in different molecules which are related to each other by the screw-axis (and also sections 2 of different molecules related by the glide-plane), so it should be on the b-axis as sections 1 and 2 of such related molecules will be rotated off the b-axis by equal amounts in the same direction.

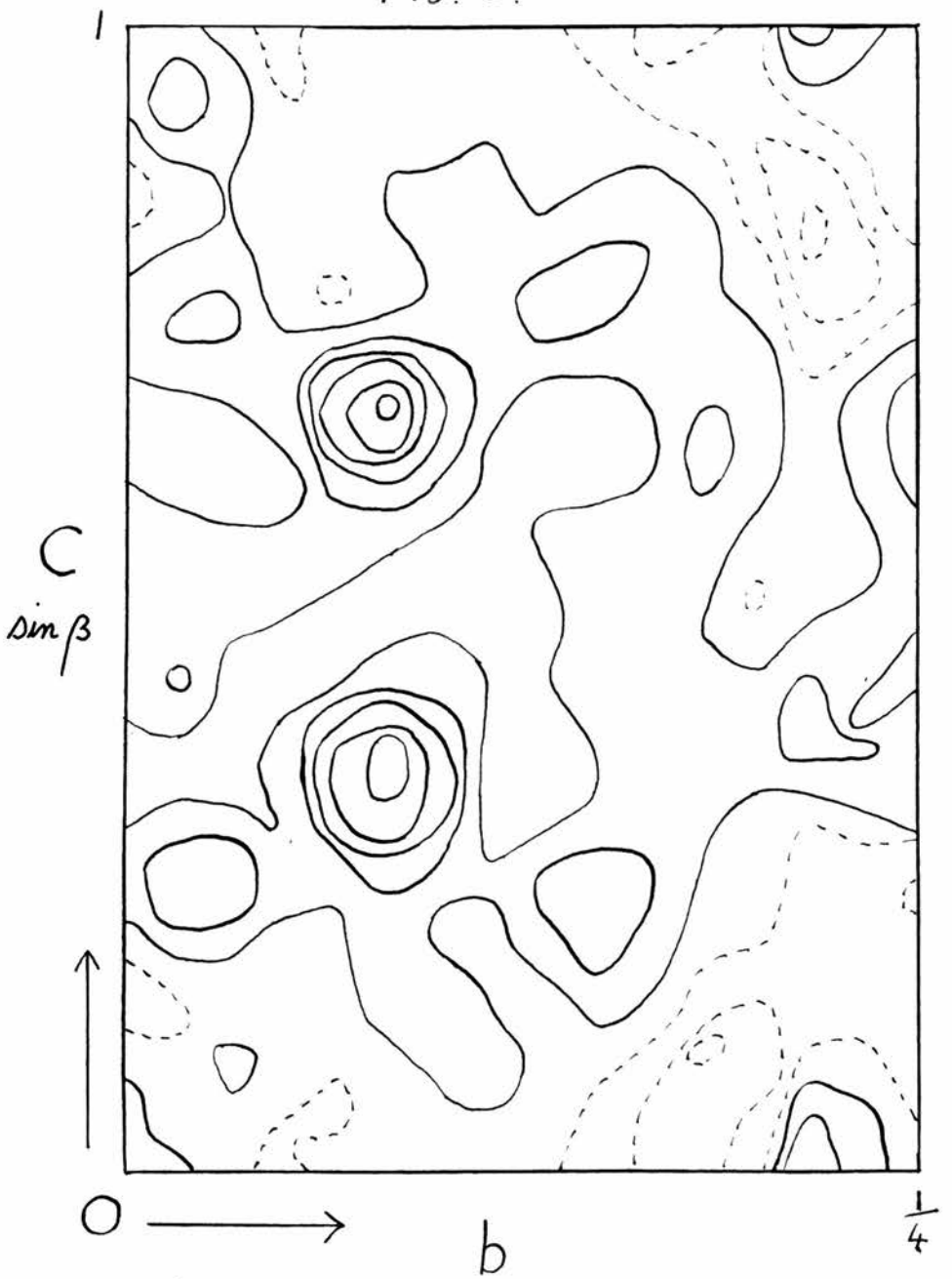
This rotation was effected and structure-factors calculated. A (Fo - Fc) map was now computed to see if the positions of the water molecules would show up. This is shown in Fig. 8. where the positions of the water molecules are clearly indicated. This was perhaps the surest confirmation of the substantial correctness of the structure. When the water molecules were included in the structure-factor calculation a disagreement factor of R = 44·4% was obtained and this refined in one cycle to 34·8% and in four further cycles to 23·9%. The refinement was carried out by (Fo - Fc) maps.

A list of the atomic parameters in this projection is given overleaf.

List of Atomic Parameters in x-axis projection.

Atom	y/b	z/c
C_1	0.420	$\overline{0.008}$
C_2	0.362	$\overline{0.210}$
C_3	0.305	$\overline{0.196}$
C_4	0.250	0.008
C_5	0.316	0.210
C_6	0.366	0.211
O_1	0.465	$\overline{0.017}$
O_2	0.408	$\overline{0.418}$
O_3	0.247	$\overline{0.392}$
O_4	0.208	0.017
O_5	0.267	0.408
O_6	0.417	0.392
O_7	0.421	0.360
O_8	0.423	0.660

FIG. 8.



$(F_o - F_c)$ MAP SHOWING POSITIONS OF H_2O MOLECULES

(c) The Problem of the Third Co-ordinate.

The essential features of the structure in two dimensions were now known, and are represented in Fig. 4 - a small rotation of each molecule about its centre being applied. This structure suggests an orientation of the molecule which is in keeping with the general characteristics of the c-axis Patterson. As was noted before, this map possesses predominant parallel striations of high ground at right angles to the b-axis, the distance between them being $\frac{b}{18}$ i.e. about 0·93Å, which is a little longer than the projection of the C - C vectors on the b-axis (0·90Å). However, from the b-axis Patterson, it may be observed that the a-axis is divided up into $\frac{a}{8}$ (i.e. 1·14Å) by somewhat similar rows of peaks, and this is a bit shorter than the C - C projection on the a-axis (1·26Å). If, then, the molecule is tilted a little to make the 1·26Å projection on the a-axis shorten to about 1·14Å, it is found that the corresponding b-axis projection lengthens to nearly 0·93Å. The required angle of this tilt is about 6°. In the b-axis projection, the average is taken including the C - O distances of 1·43Å. The distance between molecules like 1 and 2 (Fig. 4) is fixed, and distances between oxygen atoms of the type a and a^1 calculates at about 2·6Å i.e. of the right order to be a hydrogen bond and to take part in the hydrogen bonding scheme suggested earlier (P. 24) What remains to be determined is the position of molecule 1 up the a-axis with /

with respect to the centre of symmetry, and also which of the carbon atoms nearly on the b-axis is the axially substituted one (i.e. C_1). The orientation of the molecule makes the projections of $C_1 - O_1$ and $C_4 - O_4$ on the b-axis so nearly the same, that it was not possible to distinguish between the two possibilities in projection. Another factor which cannot be decided in projection is the direction in which the $C_1 - C_4$ vector slopes, and no information is given as to what way up the molecule is. Each of these possibilities if changed separately, lead to completely different structures although it was found that changing certain pairs of possibilities did not give new structures. What follows is an account of the work which led up to the complete structure determination. A number of different techniques were used. With some of them, their limitations were realised at the time; with others they were not apparent until later. What was, perhaps, the chief obstacle to an earlier solution of the structure can be summed up in a word - the waters molecules.

(i) Packing and Pattersons.

The number of possibilities for the position of the centre of the molecule in the a-axis direction seems to be limited by the three peaks at z = 0 on the b-axis Patterson. The peak nearest the origin is ruled out on packing considerations and the other two peaks are left as equally probable. When the c-axis Patterson is consulted in order to help resolve the problem, it is found that there is a large peak with a maximum at the same x-co-ordinate as the peak second nearest to the origin in the b-axis Patterson, though this peak is the lighter of the two b-axis Patterson possibilities. This peak has an x-co-ordinate of $\frac{1}{4}$, so, this being taken as the x-separation of two molecules related by a centre of symmetry, the molecules centre is put at x = $\frac{1}{8}$. In this position the only feasible orientation which will pack properly is that which, in the case of molecule 1 in Fig. 4, places C_4 on the glide plane with the x-co-ordinate of C_1 lower than that of C_4, and, of course, the x-co-ordinate of O_1 above that of C_1. Later, it was realised that the centre of the molecule was at x = $\frac{3}{8}$ and to pack properly in this position C_1 must be placed on the glide-plane with the x-co-ordinate of C_1 greater than that of C_4 and the x-co-ordinate of O_1 less than that of C_1. These structures pack very well and fit in /

in with the type of set-up suggested (P.24). Because of the experience encountered while working on the a-axis projection it was decided to use the following criterion in settling whether or not a trial-structure was worth following up.

With the co-ordinates of the trial-structure, structure factors (h k o) were to be computed excluding contributions from the water molecules whose precise positions could not be obtained in any case. Using (Fo)s signed by these (Fc)s, an (Fo - Fc) map would be calculated, and if it showed any evidence of the water molecule positions at reasonable sites, then the structure could be pursued further; if not it was to be rejected.

This proved to be a rather harsh stricture and no satisfactory structure could be wrested from the Pattersons. Other trials were made based on the peak at ($x = \frac{3}{8}$, $z = 0$) in the b-axis Patterson as the peak between two centro-symmetrically related molecules, but no acceptable solution was found. Yet the true structure is almost certainly based on one of these peaks, so it was decided to try to use some other methods first to fix a more accurate structure in which the above 'find the waters' criterion could be satisfied. The experience with the a-axis projection had shown that a fairly accurate structure (most atoms correct to within about 0·05Å) is necessary before the difference map /

map will show up the water molecules. This is because the number of electrons in the two water molecules accounts for 16% of the total number of electrons in the molecule dihydrate. But the cardinal difficulty lay in the fact that on calculating a trial structure which did not give too good agreement between the Fc's and Fo's, it was not possible to tell whether the disagreement was due to a wrong structure or simply to the exclusion of the water molecules in the Fc calculations. An (Fo - Fc) map should help to decide this point, but, as was found in the a-axis case, the test turned out to be rather a critical one.

(ii) Figure Fields and Taylor's Method.

These methods are considered together because they are based on the same principles - in fact Taylor's method may be regarded as a development of the Figure Field method - and because they both failed in this case to give the solution to the structure for the same reason.

Bragg and Lipson (23) first put forward the idea of structure factor graphs. These are contour maps representing with variation in position in the unit cell the contribution of an atom and its plane-group related counterparts, to the total structure factor of any one reflection. In the Figure Fields of Beevers and Lipson, these contours were replaced by the values of the structure factor function given at some stated interval, the interval being a particular fraction of the unit cell edge.

Such structure-factor graphs were drawn for about eight reflections of the type (h k o). This meant that a drawing of the molecule in this projection could be made on tracing paper and when fitted on the structure-factor graph in any position the value of the structure-factor for that particular reflection could be computed by adding algebraically the values of each of the atomic contributions as read off from the structure-factor graph.

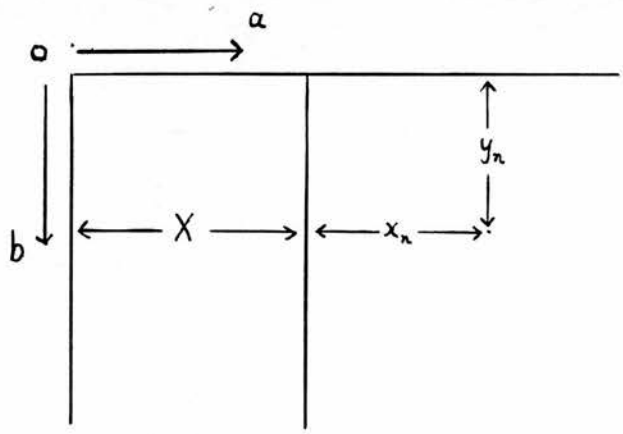
In our case, the y - and ~~x~~ z-co-ordinates were fixed, so the molecule was orientated with C_4 on the glide plane, C_1 with a lower x-co-ordinate than C_4 and O_1 with a higher /

higher x-co-ordinate than C_1. The molecule was then moved through the cell in a direction parallel to the a-axis while preserving this orientation. At any position up the a-axis, the predicted value of the structure-factor could be calculated and compared in magnitude with the Fo of that reflection. A number of reflections were chosen with Fo=0.

An attempt was made to find a position up the a-axis in which all the reflections used gave agreement between Fc and Fo.

Taylor's method, as used in this case, was really just a means of analytically finding the points along the a-axis where the calculated structure factor of the whole molecule agreed in magnitude with the Fo. The idea of the method came from the papers by Taylor (24) and Taylor and Morley (25), and the theory for the (h k o) projection which is in the plane-group p g g is developed below. It is a simplified case of that treated in (25) in that it needs only one variable viz. X.

Let $(x_n\ y_n)$ be the co-ordinates of the nth. atom of the molecule with respect to an origin arbitrarily chosen on the x-axis and let X be the co-ordinates of this origin with respect to the true origin of the unit cell (see diagram below).



Then, the combined contribution of all atoms in plane-group related molecules may be written as follows:-

(i) $\underline{h + k = 2n}$

$$G(h,k) = f_n \cos(hx+hX)\cos ky$$

$$= f_n (\cos hx \cos hX - \sin hx \sin hX)\cos ky$$

$$= f_n [(\cos hx \cos ky).\cos hX - (\sin hx \cos ky).\sin hX]$$

$$= f_n [A.\cos hX - B.\sin hX].$$

(ii) $\underline{h + k = 2n+1}$

$$G(h,k) = - f_n \sin(hx+hX)\sin ky$$

$$= - f_n (\sin hx \cos hX \sin ky + \sin hX \cos hx \sin ky)$$

$$= - f_n [(\sin hx \sin ky).\cos hX + (\cos hx \sin ky).\sin hX]$$

$$= - f_n [A^1.\cos hX + B^1.\sin hX .]$$

The theory was then applied in the subsequent manner.

The molecule was orientated as in the Figure Field case, and C_1 was given co-ordinate $x = 0$. This meant that all the atoms in the molecule could now be assigned co-ordinates (x,y) relative to the arbitrary C_1 origin, and so it was possible to evaluate functions of the type coshx, sin ky for each atom in the case of any reflection (h,k), and so values of A and B or A^1 and B^1 could /

could be readily computed for any reflection (h,k). These values are then summed for like atoms and multiplied by the value of f_n for the particular (h,k) selected, and then the contributions from the unlike atoms thus obtained are summed to give expressions of the type.

$$G(h,k) = P \cos hX \pm Q \sin hX.$$

The variation of G (h,k) with variation in X can now be easily calculated by means of Beevers-Lipson strips as shown below, the idea being to inspect G (h,k) as X varies to find at which values of X G (h,k) = Fo(h,k). This value of X will be the x-co-ordinate of C_1 relative to the true origin of the unit cell.

In practice, Fo(h,k) was usually selected with value zero, but this was neither necessary nor exclusive practice though it was convenient.

An example is given on the following page to illustrate the various steps.

Example:

Reflection (4,3), Fo = 190

$$P = \sum_{\text{Carbon}} fc \sin 4x \sin 3y + \sum_{\text{Oxygen}} fo \sin 4x \sin 3y$$

$$= (10+2+2+\overline{10}+1+1) \times 3{\cdot}0 + (\overline{6}+\overline{9}+\overline{6}+\overline{6}+\overline{10}+\overline{7}) \times 4{\cdot}8$$

$$= \overline{193}.$$

$$Q = \sum_{\text{Carbon}} fc \cos 4x \sin 3y + \sum_{\text{Oxygen}} fo \cos 4x \sin 3y.$$

$$= (\overline{1}+\overline{4}+\overline{4}+\overline{3}+3+6) \times 3{\cdot}0 + (2+\overline{5}+\overline{8}+\overline{4}+3+7) \times 4{\cdot}8$$

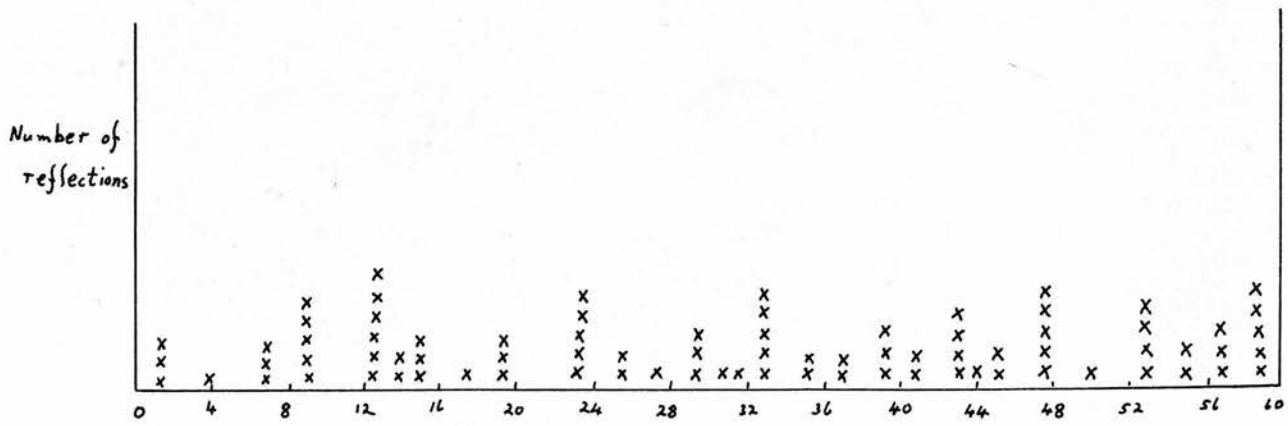
$$= \overline{32}.$$

X in $^1/_{60}$ths

P or Q		h	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
$\overline{193}$	CE	4	$\overline{193}$	$\overline{176}$	$\overline{129}$	$\overline{60}$	20	96	156	189	189	156	96	20	$\overline{60}$	$\overline{129}$	$\overline{176}$	$\overline{193}$	($\overline{194}$)
$\overline{32}$	SE	4	0	$\overline{13}$	$\overline{24}$	$\overline{30}$	$\overline{32}$	$\overline{28}$	$\overline{19}$	$\overline{7}$	7	19	28	32	30	24	13	0	(0)
	G(h,k)		$\overline{193}$	$\overline{189}$	$\overline{153}$	$\overline{90}$	$\overline{12}$	68	137	182	196	175	124	52	$\overline{30}$	$\overline{105}$	$\overline{163}$	$\overline{193}$	

So it is seen that for reflection (4,3), G(h,k) = Fo(h,k) = 189 where X has a value of $^2/_{120}$ or $^{15}/_{120}$.

This procedure was carried out for about 33 reflections and a plot was made of the number of reflections indicating a particular value of X against X. This plot is shown below.



No unequivocal value of X is indicated, but X = $^{9}/_{120}$ths. and $^{13}/_{120}$ths. were tried as values of the x-co-ordinate of C_1. Neither of these trials satisfied the 'find the waters' criterion.

The reason that the Figure Field method and Taylor's method failed would appear to be that the calculated value of C(h,k) excluding the water molecules from the calculation, is not accurate enough to be of any use. Added to this was the fact that in the correct position of the molecule (as later determined) O_1 and O_4 were in different orientations to these used in the above calculations. Even the cumulative evidence from 33 reflections did not afford an accurate enough X value to give a useful (Fo - Fc) synthesis. If there had been no accompanying water of crystallisation, it seems likely that Taylor's method would have been ideal for the situation where it can be applied in the simplified form above.

(iii) Generalised Patterson Projections.

Having failed to obtain the x-co-ordinates for the molecule by Taylor's method, it was decided to try yet another technique which uses the knowledge gained from the solved projection to obtain the elusive x-co-ordinates. The method was that of Generalised Patterson Projections.

Corresponding to the electron density distribution

$$\rho(x,y,z) = \frac{1}{V} \sum \sum \sum F(h,k,l) \exp\left[-2\pi i (hx+ky+lz)\right]$$

it is possible to define the generalised projection of this distribution on a plane perpendicular to the a-axis as the distribution modulated by some operator such as $\exp\left[2\pi i\, Hx\right]$ so,

$$\rho_H(y,z) = a \int_o^1 \rho(x,y,z) \exp\,(2\pi i.Hx)\, dx.$$

Now, $\int_o^1 \exp\left[2\pi i.(H-h)x\right] dx \neq 1$ if $H = h$

$= 0$ if $H \neq h$

So $\rho_H(y,z) = \frac{1}{A} \sum_k \sum_l F(H\ k\ l) \exp\left[-2\pi i(ky+lz)\right]$

after Cochran and Dyer (26).

It may be noted that when $H = 0$, this becomes

$$\rho_o(y,z) = \frac{1}{A} \sum_k \sum_l F(o\ k\ l) \exp\left[-2\pi i(ky+lz)\right]$$

$$= \rho(y,z)$$

So in using the F(o k l)'s in the Fourier, we obtain the ordinary projection parallel to the a-axis, but using F(H k l)'s results in the generalised projection which is an electron distribution with peaks in the same places as the /

the F(o k l) synthesis, but with peak heights modulated by the operator exp(2 π i Hx). In the ordinary projections the Fourier series representing the electron density is everywhere positive. However, in the case of the generalised projections the peak modulation may result in positive density being converted into its negative equivalent and in this way symmetry elements of the reversal type which we previously came upon in dealing with the Partial Patterson (Fig. 6), may once again be encountered.

The idea may be extended to Patterson Fourier series and this application was first made by Dyer (27). Consider the generalised Patterson Fourier

$$P_{H(y,z)} = \frac{1}{A} \sum_{k} \sum_{l} |F_{Hkl}|^2 \cos 2\pi (ky + lz)$$

Let $P_o(y,z)$ and $P_1(y,z)$ denote the Patterson functions for H = 0 and H = 1 respectively. Then, if a peak at (y,z) in $P_o(y,z)$ is taken as of unit height, there will be a peak at the same position in $P_1(y,z)$ of height cos $2\pi.x_r$ where x_r is the x-value of the separation between the two electron densities separated by the vector (y,z) in the a-axis projection.

Triple-film overlay Weissenberg photographs were taken about the a-axis of the first layer line, and the intensities corrected in the usual manner by the Lorentz and polarisation factors to give $F^2(_1$ k l) rel. and these were multiplied /

multiplied by exp (2·6 sin θ) to give peak sharpening. Using these values, the generalised Patterson function viz.

$$P_{(i,y,z)} = \sum_{k} \sum_{\ell} \left[(F^2_{ik\ell} + F^2_{ik\bar{\ell}}) \cos ky \cos \ell z - (F^2_{ik\ell} - F^2_{ik\bar{\ell}}) \sin ky \sin \ell z \right]$$

was computed (Fig. 18), using Beevers-Lipson strips and a Hägg-Laurent analogue computer.

To determine the value of x_r above, the heights of corresponding peaks on $P_0(y,z)$ and $P_1(y,z)$ were measured. These heights could not be compared directly for two reasons.

(i) No precautions had been taken to ensure that the intensities on the two layer-lines were on the same scale.

(ii) The peak heights on upper layer-line projections are always less than those on the zero layer-line because the effective atomic scattering factors and hence the coefficients $F^2(h\ k\ l)$ decrease with increasing H(i.e. increasing sin θ).

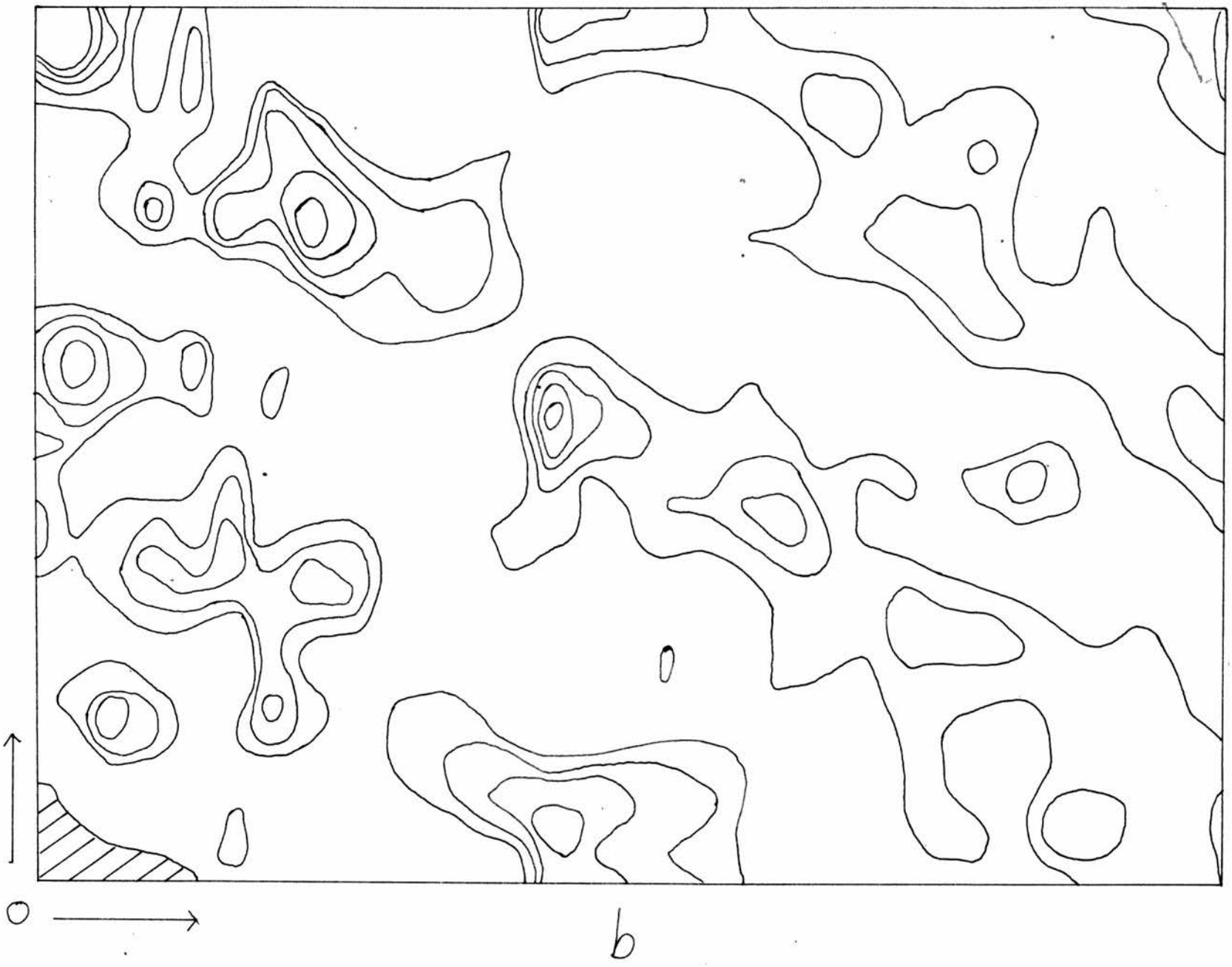
To obviate these effects, the ratio

$$\alpha = \frac{\text{Height of peak}}{\text{Height of origin peak of corresponding projection.}}$$

was determined for each peak used, the denominator of α being determined only by the scaling-factor and the sin θ value.

This ratio can now be used to compare first layer-line and zero layer-line peaks just as Dyer (27) used it to compare second and first layer-line peaks.

Then /



GENERALISED PATTERSON $P_1(vw)$

FIG. 18.

Then $\frac{\alpha_1}{\alpha_0} = \cos 2\pi . x_r$ and in each case $\frac{\alpha_1}{\alpha_0}$ may be evaluated from the Patterson maps $P_1(y,z)$ and $P_0(y,z)$.

The method was used first to measure some of the x-components of the intra-molecular vectors thus affording a check on the orientation of the molecule as indicated by the zero-layer-line projection.

A comparison was then made of Patterson peaks given by molecules related by the centre of symmetry and the screw axis. In the case of the centre of symmetry, the value of x_r obtained should be twice the x-distance of the centre of the molecule above the centre of symmetry. With the Harker peak, the information given will be the x-distance of the molecular centre from the screw-axis at $x = \frac{1}{2}$. When the calculations were done, both the centre of symmetry peaks and the Harker peaks indicated that the centre of the molecule should be at $x = \frac{17}{120}$ above the centre of symmetry. The molecule was placed in this position, but the resulting (Fo - Fc) map did not succeed in locating the water molecules excluded from the Fc calculations.

A few points may be noted about this part of the work. First, the value of the x-co-ordinate indicated for the centre of the molecule is nearly the same as that preferred by the Figure Field method. This would seem to point to the second feature which is that the whole trouble now appeared to be the lack of precision given /

given by the methods applied. The (Fo - Fc) map is only useful if, as indicated earlier nearly all the atoms of the molecule are within 0·05Å of their correct positions. A third point, specific to the method of Generalised Projections, is that when this technique is being applied, every peak used in the Pattersons must be amenable to labelling as due to definite vectors. If overlapping occurs, as it does in the case of inositol, the method is only applicable if the overlapping vectors are parallel to one another, so that they have identical x_r values. This was also the case in inositol, but where this situation does not obtain, the operator modulating the peak heights in the P_H maps will be a compound quantity not readily resolvable.

(iv) Direct Methods.

The connotation 'direct' is usually reserved for methods which attempt to derive the phases of the structure-factors directly by mathematical means from the X-ray diffraction data. The limitations of these methods are such, of course, that only a fraction of the structure-factors can be given signs (in the centro-symmetrical case), but for our purpose, it was hoped that a Fourier synthesis using such signs as could be obtained, might give an indication of the x-co-ordinates of the molecule. Two methods were applied viz. Harker - Kasper inequalities and Sayre's sign relationships. Formal derivations of the expressions used in these methods have been given by Woolfson (28) and we will confine ourselves to a brief description of the way in which they were applied.

Since myo-inositol dihydrate crystallises in a centro-symmetrical space-group, the problem reduces itself to that of determining the signs of the various structure-factors whose magnitudes have been derived from the intensities. The (h k o) projection was selected for investigation.

For the application of direct methods it is convenient to scale the F's so that the maximum value of F(h k o) is unity. The terms used are defined as follows:- /

follows:- $n_j = \frac{f_j}{\sum_1^N f_j} = \frac{f_j}{F(0,0,0)}$. $\overline{U^2} = \sum_1^N n_j^2$. $\frac{U^2(hkl)}{\overline{U^2}} = \frac{I(hkl)}{\overline{I}}$

$$\therefore S_o, \quad U(h,k,l) = \sqrt{\frac{I(h,k,l)}{\overline{I}}} \cdot \sqrt{\overline{U^2}}$$

$$= F(h,k,l) \cdot \frac{(\overline{U^2})^{\frac{1}{2}}}{F}$$

$$= \underline{F(h,k,l) \cdot S}.$$

It is clear that U(h k l), the so-called unitary structure-factor, will satisfy the condition $1 \geqslant U(hkl) \geqslant -1$, so as a first step in applying direct methods, the F(hkl)'s are converted to U(hkl)'s.

To do this, the $F_o(hk0)$'s were divided up into seven zones of sin θ and for each zone values of $S = (\overline{U^2})^{\frac{1}{2}} \cdot \overline{F}^{-1}$ were calculated. $(\overline{U^2})^{\frac{1}{2}}$ was evaluated as follows.

Take inositol molecule as C_6O_8.

Then $\sum_1^N f_j = 6 \times 6 + 8 \times 8 = 100$,

so for unit cell (4 molecules), $\sum_1^N f_j = 400$

$$\therefore \overline{U^2} = \sum_1^N n_j^2 = 4 \times 6 \left(\frac{6}{400}\right)^2 + 4 \times 8 \left(\frac{8}{400}\right)^2.$$

$$= 1{\cdot}82 \times 10^{-2}$$

$$\therefore \underline{(\overline{U^2})^{\frac{1}{2}} = 1{\cdot}35 \times 10^{-1}}.$$

The values of $S = (\overline{U^2})^{\frac{1}{2}} \cdot F^{-1}$ thus obtained for each zone were plotted against sin θ. The mean value of sin θ for each zone, $\overline{\sin \theta}$, is not quite the same as the mean of the two extreme values of the range, but it was used /

used as a close enough approximation. A straight line graph was obtained. The individual values of $F_o(hk0)$ were then converted to U(hk0)s by multiplying by the appropriate value of $(\overline{U^2})^{\frac{1}{2}}$. F^{-1} as read off from the graph. The U(hk0)'s thus calculated are shown in Table / .

The inequalities employed were of the Harker - Kasper type.

$$[|U(\underline{h})| + |U(\underline{h}')|]^2 \leqslant [1 + S(\underline{h}).S(\underline{h}').S(\underline{h}+\underline{h}').|U(\underline{h}+\underline{h}')|] \times [1 + S(\underline{h}).S(\underline{h}').S(\underline{h}-\underline{h}').|U(\underline{h}-\underline{h}')|] \quad \ldots\ldots (a)$$

where S(h) represents the sign of U(h).

In order that a start may be made, the fact that certain reflections may be arbitrarily assigned signs is used. This possibility arises from the fact that there are, in a centrosymmetric space-group, the various centres of symmetry any of which may be selected as the origin of the axes of reference. In our case there are four such centres (in projection) and so two reflections, one of which has k = odd and the other of which has h = odd, may be arbitrarily signed. Since the inequality relationships can be most fruitfully applied to reflections with large U(h)values, a sign was given to the (4,3,0) and the (7,4,0) reflections. The theoretical basis for this arbitrary sign assignment depends on so-called 'structure invariants' and is given by Woolfson (op. cit. p. 21ff.).

Another simpler relationship used to start off the signing is that if the two reflections h and 2h have both /

L I S T OF U(h,k,o)s X 100.

h \ k	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	h
10	8	14	8	8	15	29	30	23	38														10
9	0	0	0	26	13	33	20	28	0	14	15	9											9
8	0	7	0	12	37	0	31	13	0	8	8	0	14	15	0								8
7	0	10	0	6	59	0	0	7	0	7	31	13	0	14	35	0	9						7
6	9	17	36	0	41	0	30	6	6	16	11	23	31	13	33	14	0	0	16				6
5	0	14	0	4	12	20	5	5	5	6	6	21	7	24	8	13	14	8	9	16			5
4	8	14	6	46	9	14	0	15	8	13	9	6	15	28	12	0	8	0	0	9	15		4
3	0	18	3	3	8	26	20	33	7	5	17	0	10	10	28	18	12	8	14	8	15	0	3
2	17	32	30	5	22	17	5	31	4	33	11	21	9	6	26	7	30	52	13	24	11	0	2
1	0	5	13	14	12	14	15	13	23	0	15	8	5	14	0	11	0	31	8	35	36	23	1
0	-	0	8	0	2	0	57	0	15	0	7	0	53	0	15	0	7	0	86	0	9	0	0
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	

k

TABLE I

both high values of U($\underline{h}$), then S($2\underline{h}$) is probably positive. This criterion is applied to give the (8,6,0) reflection a + sign.

The U(hk0)'s were now plotted out on a reciprocal net (four quadrants) on a piece of white paper and an identical plot was made on a piece of tracing paper which could conveniently be overlaid on the first net. This provides a ready method of locating reflections related by $\underline{h}$, $\underline{h}^1$, ($\underline{h}+\underline{h}^1$) and ($\underline{h}-\underline{h}^1$). If any reflection is selected as $\underline{h}$, the origin of the upper net can be moved to this reflection's position on the lower net (while preserving the relative orientations of both nets). Then, if any reflection $\underline{h}^1$ is selected on the upper net, the reflection $\underline{h}+\underline{h}^1$ will be directly below it on the lower net and similarly the reflection $\underline{h}-\underline{h}^1$ will be below $-\underline{h}^1$.

Two examples of the application of the inequalities are given below.

(i) The reflection (4,3,0) is taken as $\underline{h}$ and ($\bar{3}$,7,0) as $\underline{h}^1$. Applying the above construction to locate ($\underline{h}+\underline{h}^1$) and ($\underline{h}-\underline{h}^1$), it is seen that

$$U(\underline{h}) = +46,\ U(\underline{h}^1) = 33,\ U(\underline{h}+\underline{h}^1) = 15,\ U(\underline{h}-\underline{h}^1) = 59.$$

Applying the inequality (a)

$$(0{\cdot}46+0{\cdot}33)^2 = 0{\cdot}63 \leqslant (1 \pm 0{\cdot}15)(1 \pm 0{\cdot}59).$$

Clearly, the signs S($\underline{h}^1$), S($\underline{h}+\underline{h}^1$) and S($\underline{h}-\underline{h}^1$) will determine which of the $\pm$ possibilities hold. However, /

However, if both possibilities were -, the inequality would be destroyed

i.e. $0{\cdot}63 \not\leqslant 0{\cdot}85 \times 0{\cdot}61$.

No further information can be gained.

(2) $\underline{h} = (7,4,0)$, $\underline{h}^1 = (2,1,0)$

Then $U(\underline{h}) = 59$, $U(\underline{h}^1) = 32$, $U(\underline{h}+\underline{h}^1) = 33$, $U(\underline{h}-\underline{h}^1) = 4$.

Applying inequality (a)

$$(0{\cdot}59 + 0{\cdot}32)^2 = 0{\cdot}83 \leqslant (1 \pm 0{\cdot}33)(1 \pm 0{\cdot}04).$$

Again it may be noted that both possibilities may not be - simultaneously. However, more information is forthcoming, because, even if the second bracket $[1 \pm 0{\cdot}04]$ does provide its maximum contribution viz. 1·04 to the R.H.S. of the inequality, the first sign must still be + to satisfy the inequality i.e. under no circumstances can the first bracket sign be - .

This means that $S(\underline{h})$. $S(\underline{h}^1)$. $S(\underline{h} - \underline{h}^1) = +$ i.e. $S(\underline{h}^1)$. $S(\underline{h} - \underline{h}) = +$ and so it is seen that $S(\underline{h}^1) = S(\underline{h}-\underline{h}^1)$. Both may be given the same unknown sign a.

In this way various relationships between the signs may be established and many of them afford unequivocal signs to reflections. In the above case, many other reflections may prove to have sign a, and at a later stage it may transpire that $a = +$, so one can go back and give a + sign to all these reflections proved to be a. The reflections with large $U(\underline{h})$ values are usually most readily signed.

Using /

Using these inequalities, signs were given to about 10 reflections.

Attention was then directed to the use of sign-relationships. In this case use was made of the fact that $S(\underline{h}) . S(\underline{h^1}) . S(\underline{h}+\underline{h^1}) \approx +$

This may be derived both from inequalities and from Sayre's equation

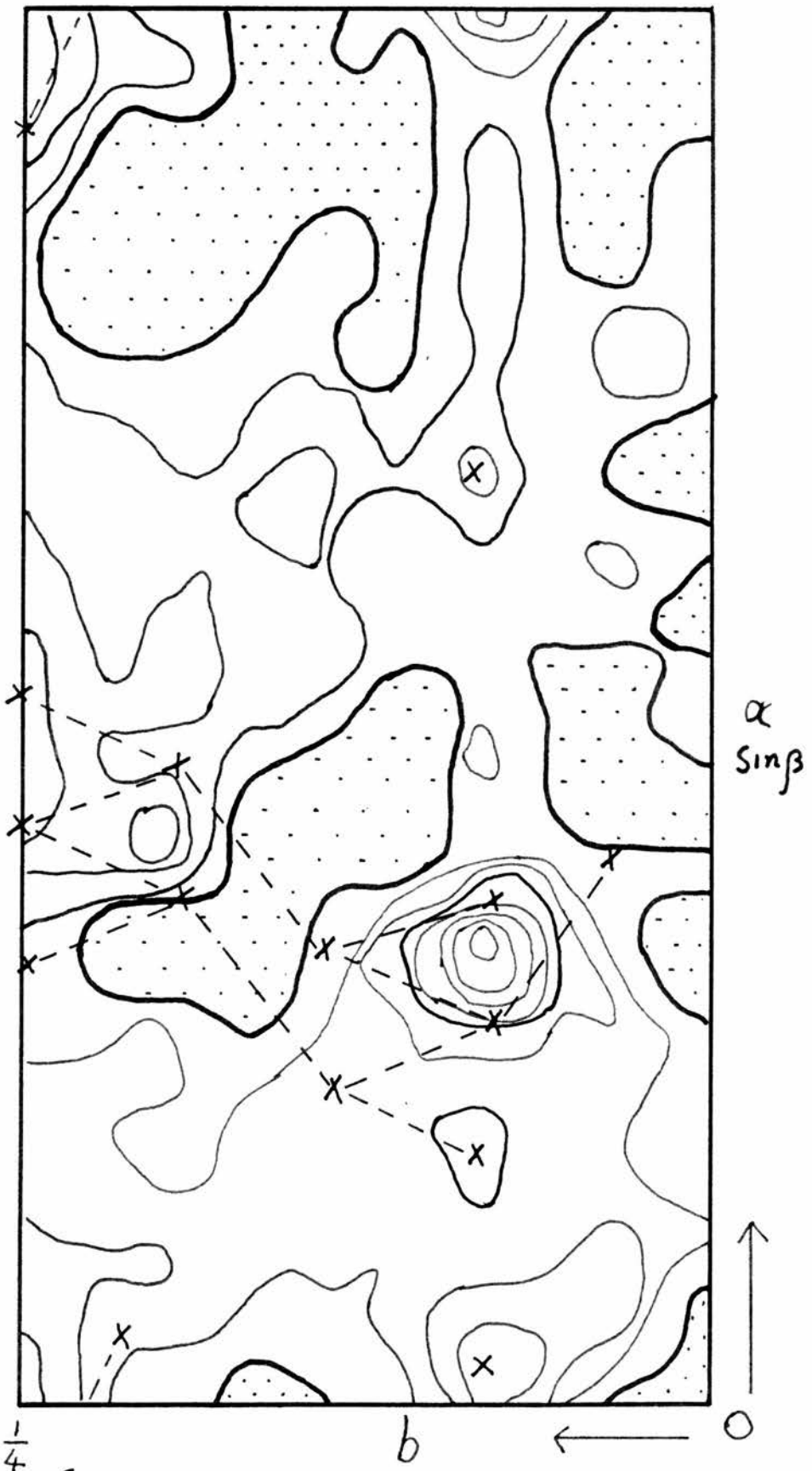
$F_h = \frac{\theta_h}{V} \sum_{h^1} F_{\underline{h1}} . F_{\underline{h}-\underline{h1}}$ where θ_h is a function of the sin θ value of $F_{\underline{h}}$, and it is applicable only when the values $F_{\underline{h}}$, $F_{\underline{h1}}$ etc. are large.

For each reflection $\underline{h}$ selected, as many sign relations as possible were searched for. If more than about eight such relations were found and if more than two thirds of these relations indicated the same sign for $\underline{h}$, then that indication was accepted and used in the search for other signs. So it is essentially a probability method.

In this way about one third of the (h k o) reflections were given signs and a Fourier synthesis was constructed using these signed structure-factors. This is shown in Fig. 9. and what was thought to be the best fit of the molecule preserving the known y-co-ordinates, is shown superimposed on the map. The water molecules were included in the calculations.

An /

FIG. 9.



FOURIER FROM DIRECT METHODS

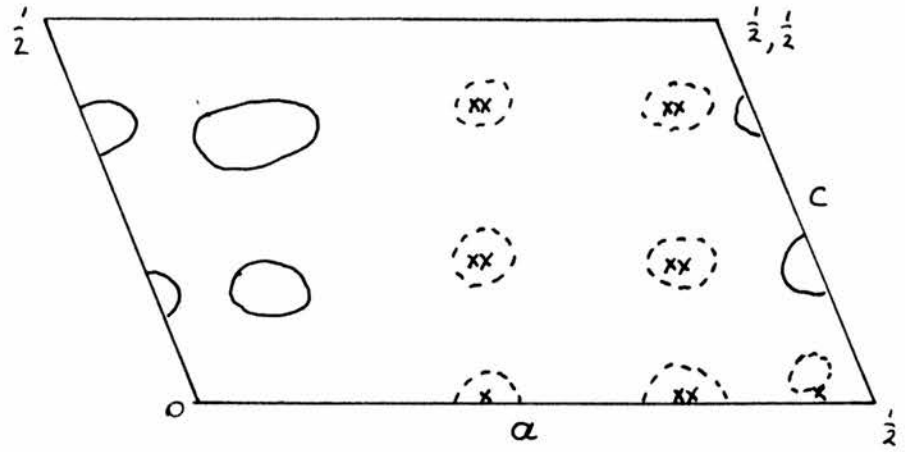
An attempt made to refine this structure by Difference synthesis was not successful, and yet the position of the centre of the molecule is almost on what later turned out to be the correct position, but the axial hydroxyl group is at the wrong end of the ring. The failure of the structure to give intelligible Difference maps must have been due to the general inaccuracy of the structure and the proximity of some of the annular atoms to one another in this projection.

(v) Three-dimensional Patterson.

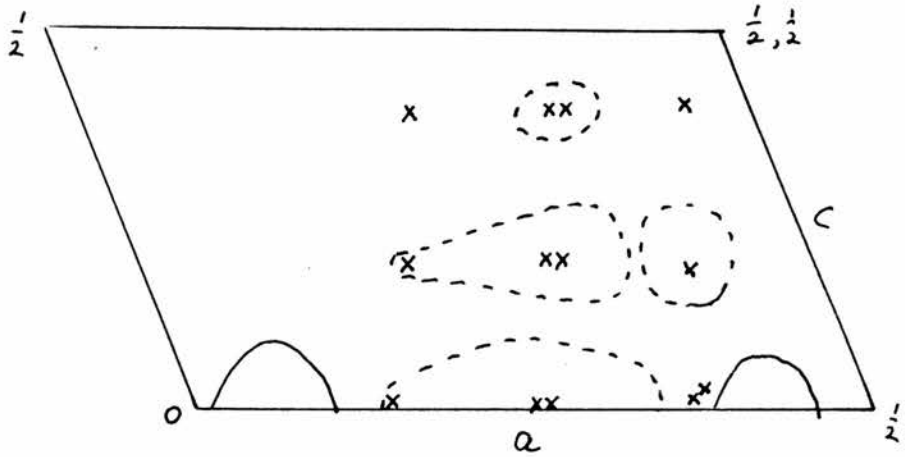
Before going on to describe the three-dimensional work it may be as well to mention briefly a Difference Map study that was carried out. This is illustrated in Fig. 10. Preserving the orientation of the molecule as in section 2c(ii), Difference maps were calculated for various positions of the centre of the molecule up the a-axis. In Fig. 10, the atomic positions are marked with crosses, the negative regions (below about 4 electrons per $\mathring{A}^2$) are shown in broken contours and the positive regions (above 4 electrons per $\mathring{A}^2$) in full line contours. It is clear from the maps obtained that the molecule centre at x = 15/120ths. appears to be the most satisfactory. The true position later showed up as 45/120ths. i.e. $\frac{x}{4}$ from this. The structure did not refine.

It was decided now to compute a three-dimensional Patterson synthesis with a view to finding the x-parameters of the atoms in the molecule. The three-dimensional data was obtained from a series of triple-film overlay Oscillation photographs taken about the c-axis. Eighteen ten degree Oscillation pictures were taken about this axis and this enabled all the reflections in the appropriate tore swept out in the reciprocal sphere to be recorded. The layer-lines on the photographs only went up to l = 3, but high order h and k reflections were obtained. Some more /

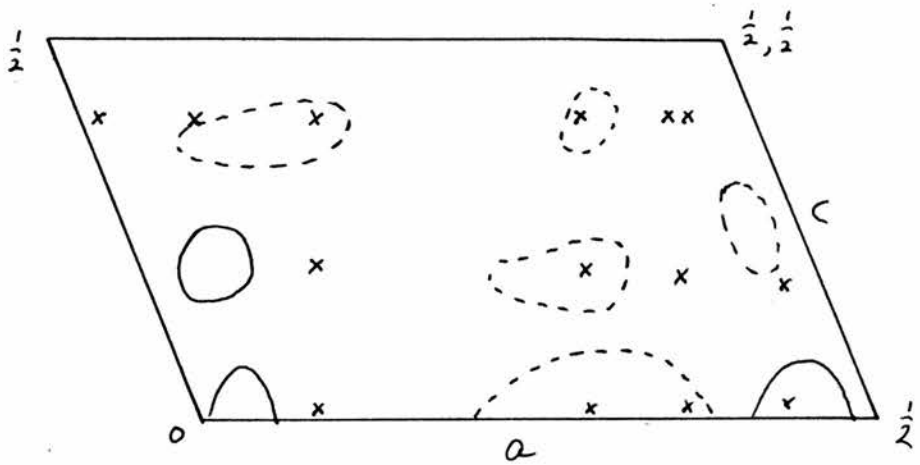
DIFFERENCE MAP STUDY FIG. 10.



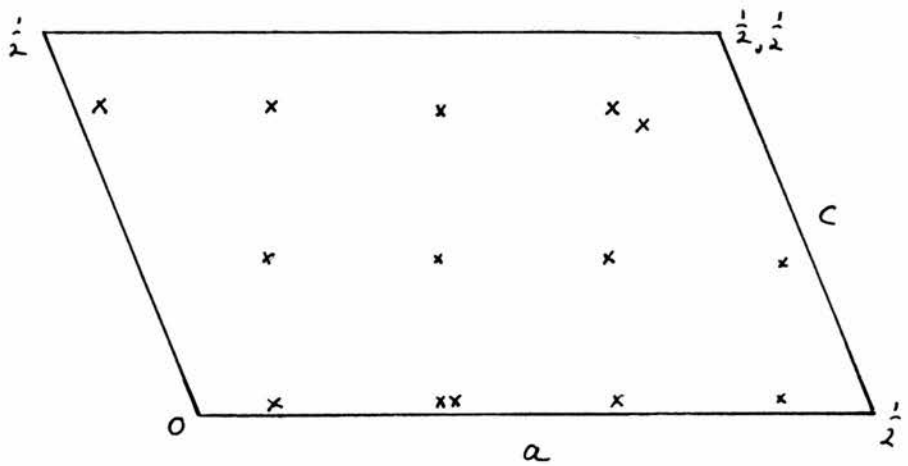
CENTRE
AT ~ $\frac{30}{120}$ H_s.
(NO WATERS IN)



CENTRE
AT ~ $\frac{23}{120}$ H_s.
(NO WATERS IN)



CENTRE
AT ~ $\frac{17}{120}$ H_s.
(WATERS IN)



CENTRE
AT $\frac{15}{120}$ H_s.
(WATERS IN)

more low order reflections were recorded on photographs about the a-axis and these were included in the three-dimensional data which is listed after p. 82.

The justification for doing a three-dimensional Patterson synthesis with what amounted to about 60% of the possible number of reflections was as follows. The reflections omitted from the synthesis were all of order $l > 3$. Since it was the x-parameters which were sought, the important high-order h reflections were nearly all included, and the lack of high-order l reflections would merely result in a lack of definition of the peaks in the synthesis along the c-axis, and accurate information was already to hand on the c-axis parameters. The a-axis positions, however, (and b-axis positions) should be well defined.

The sections of this three-dimensional Patterson function which proved most useful in solving the structure are shown in Figs. (11 - 17) and the streaking of the peaks along the c-axis direction is very marked.

The $y = \frac{0}{10}$ section shows the 2·4Å ring vector at right angles to the a-axis as was known from the a-axis projection work, but the most illuminating section is that at $y = \frac{20}{60}$ because it is clear that the peak at $x = \frac{1}{2}$, $z = 0$ is the peak from the vector between two molecules related by a centre of symmetry. The environment of this peak is similar to that of the origin and it was a close study of /

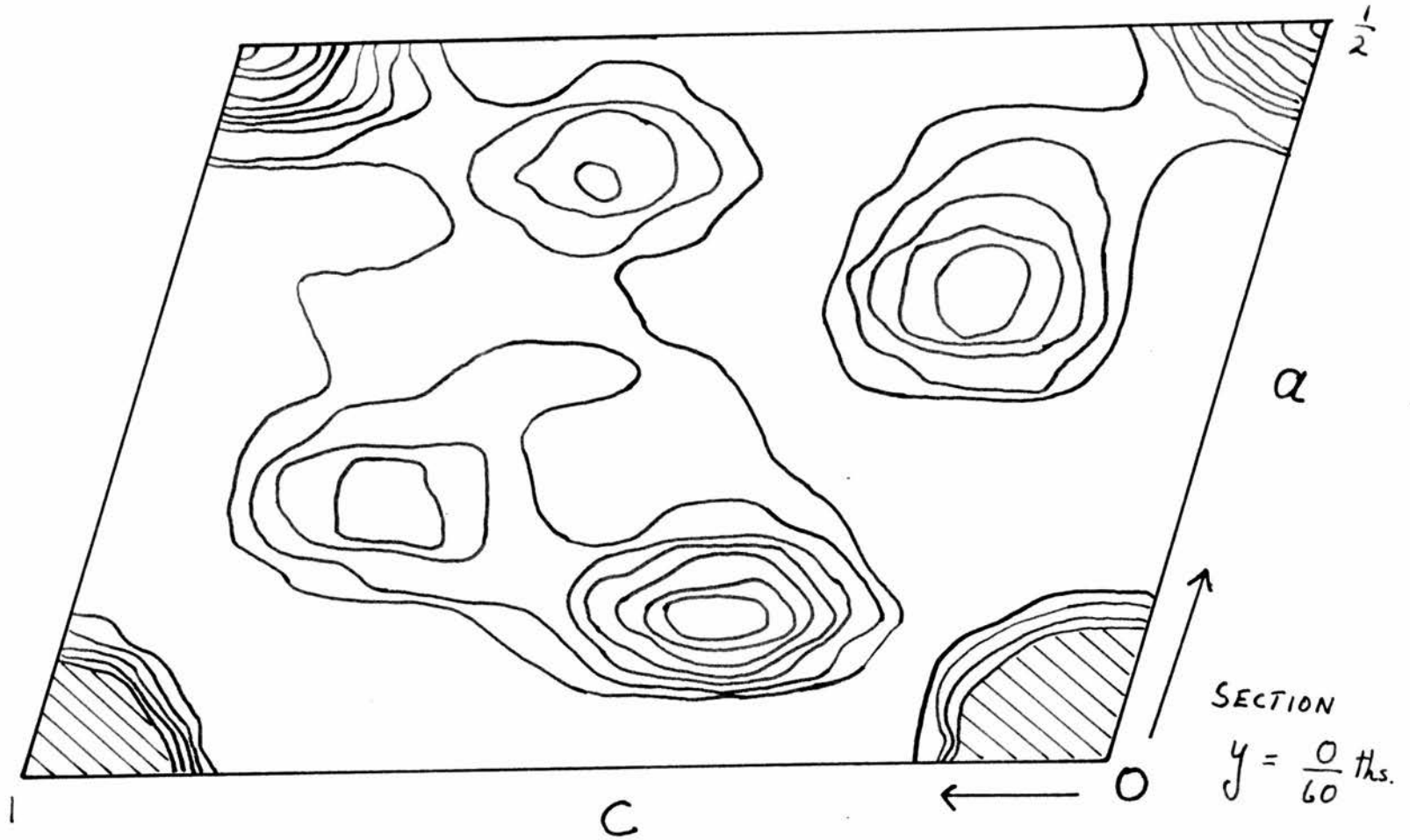


FIG. 11.

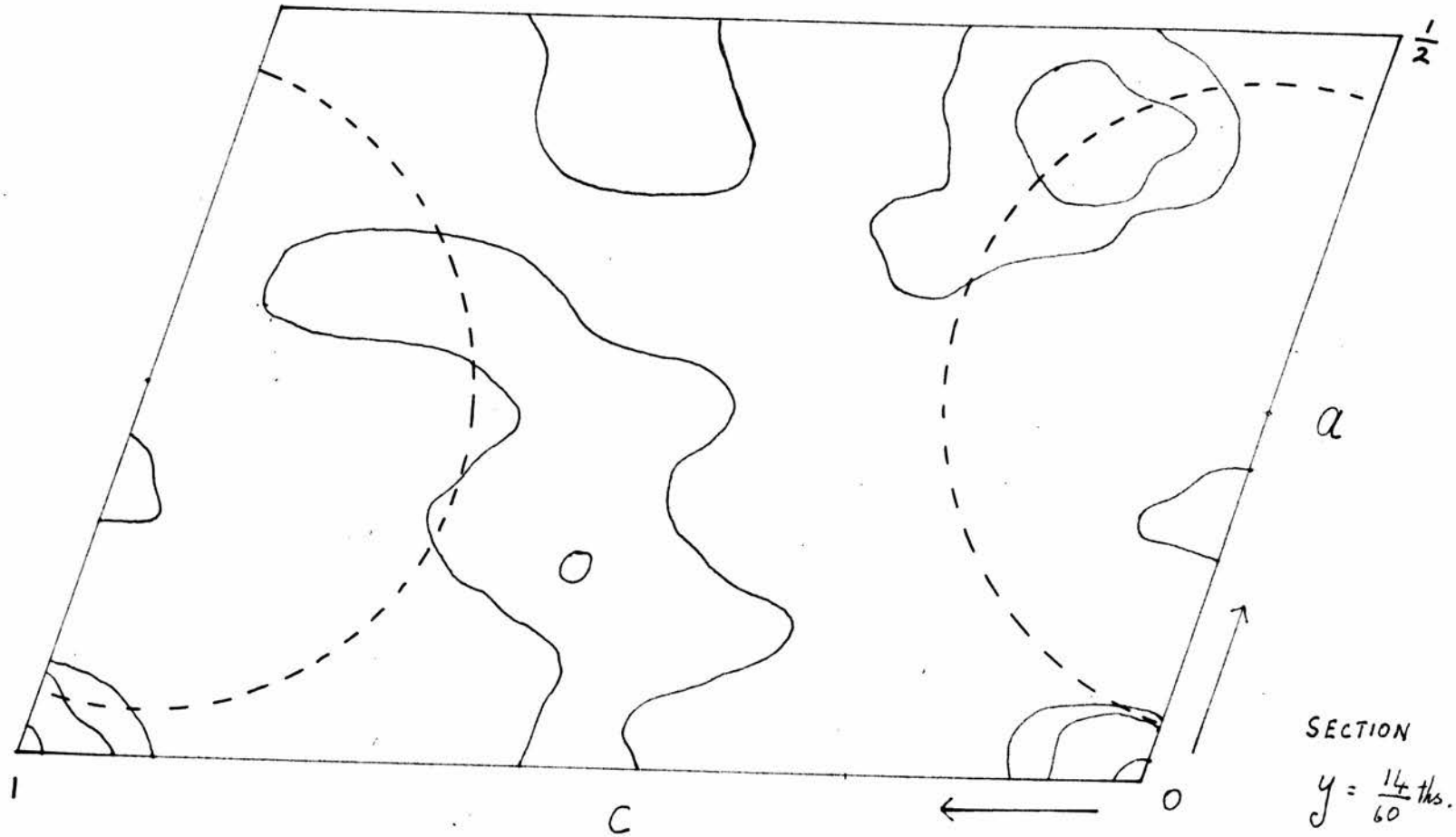


FIG. 12.

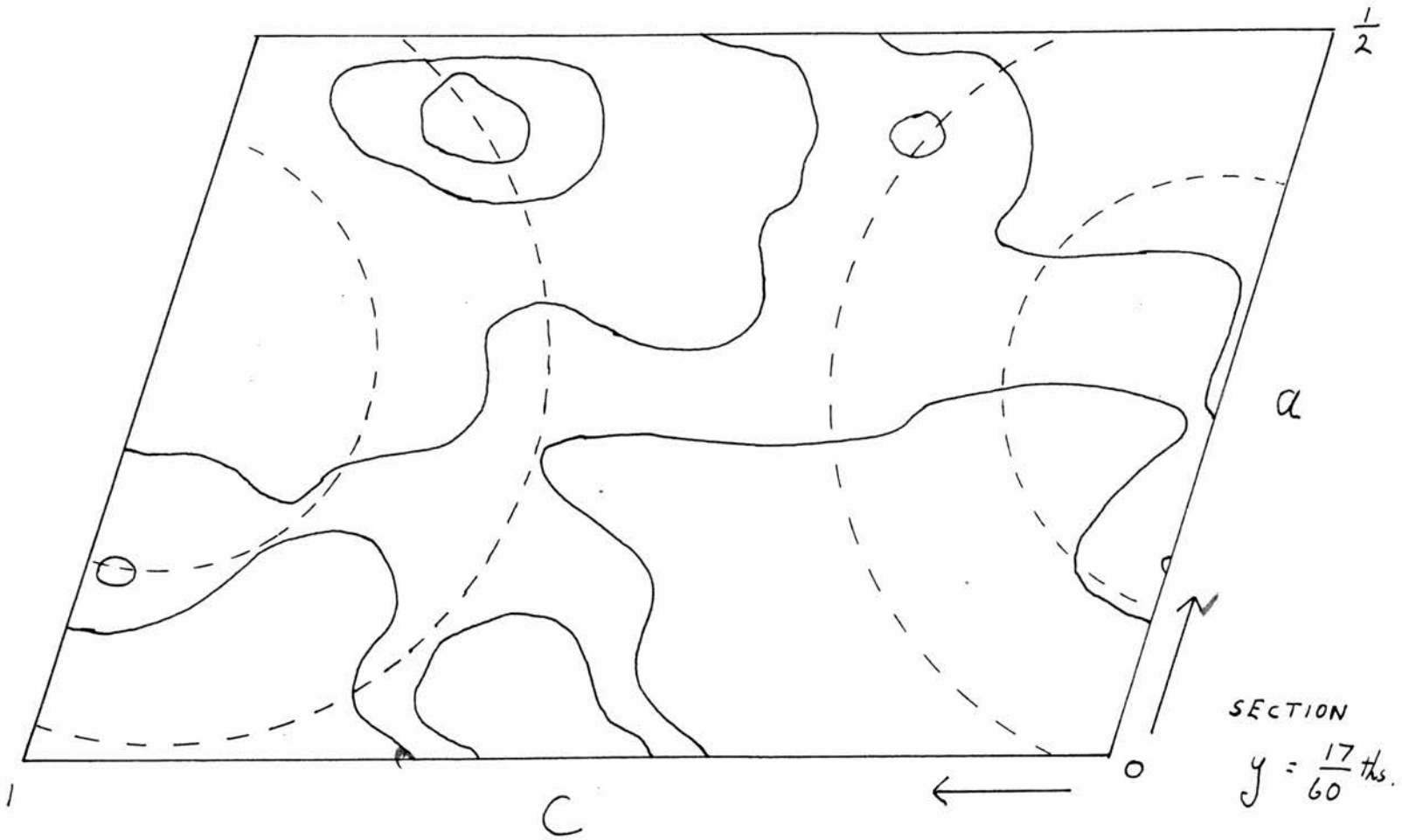


FIG. 13.

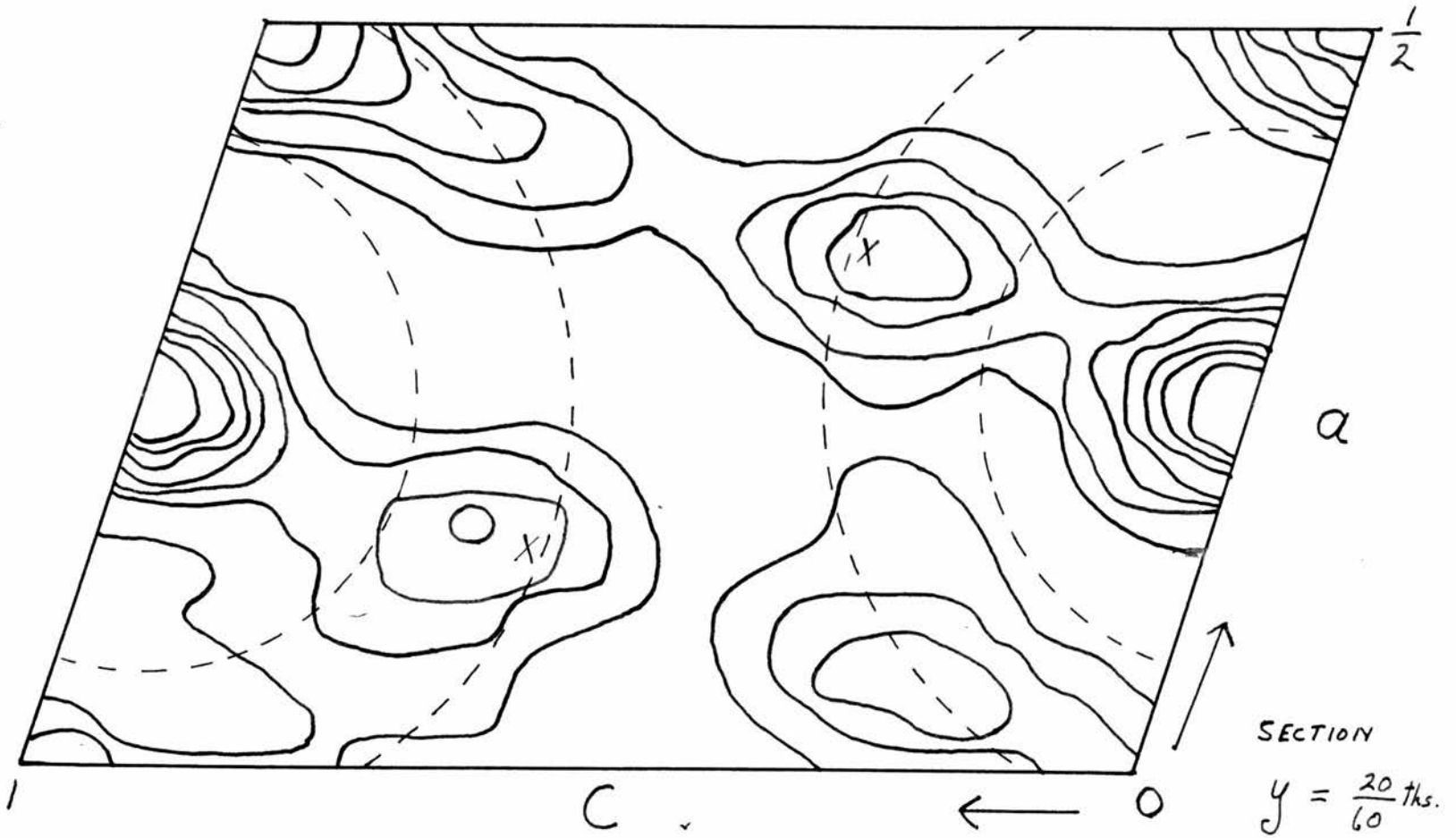


FIG. 14.

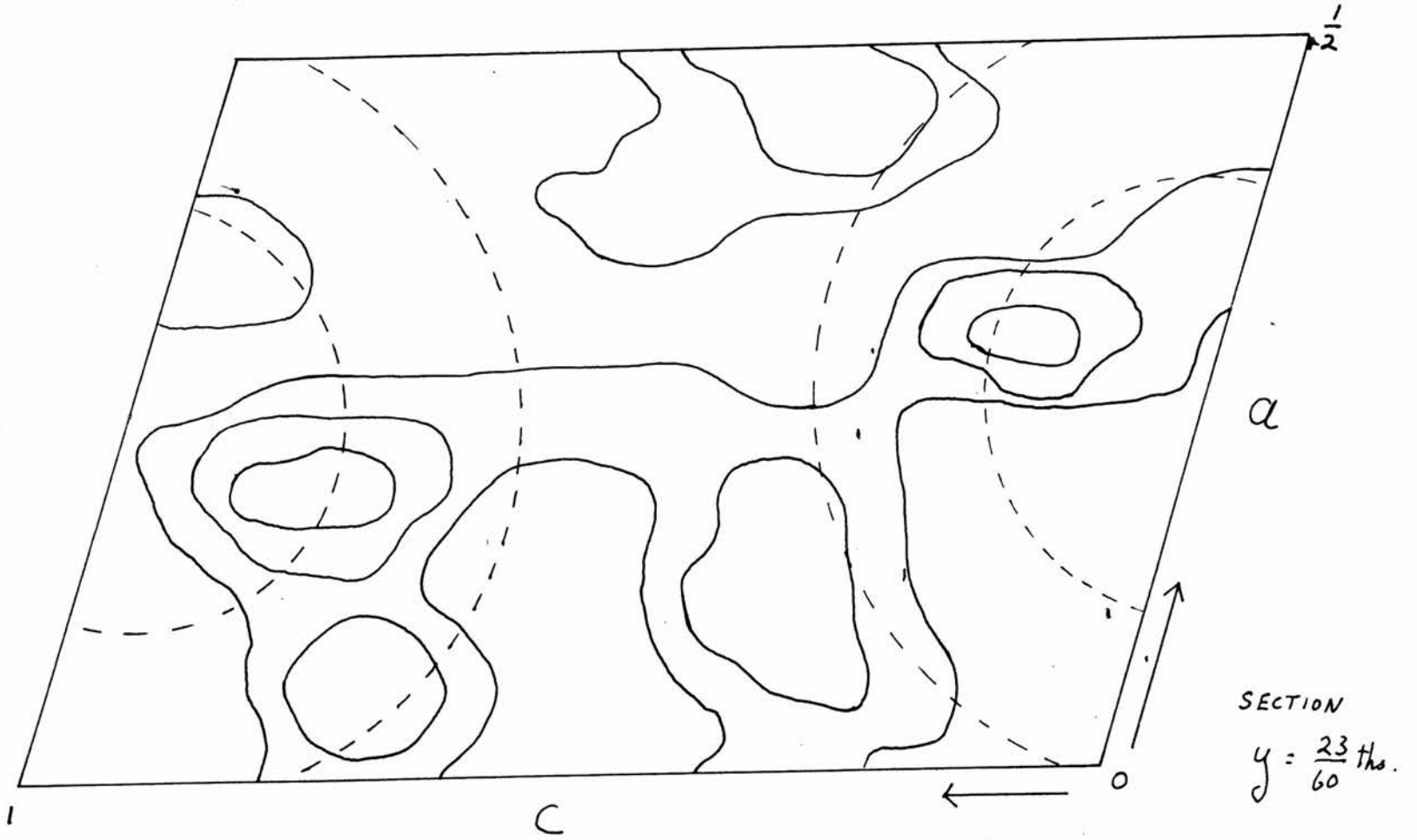


FIG. 15.

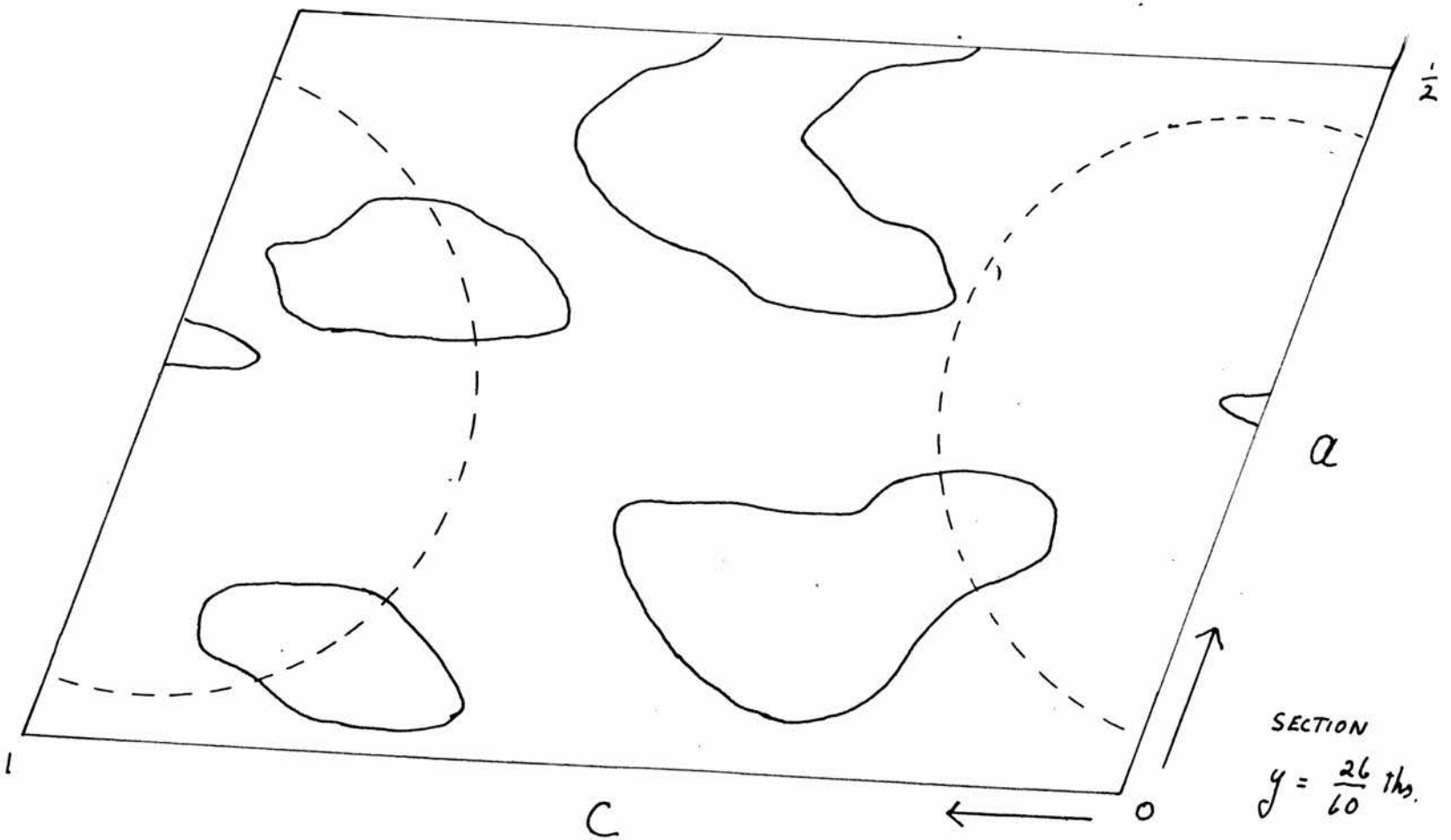


FIG. 16.

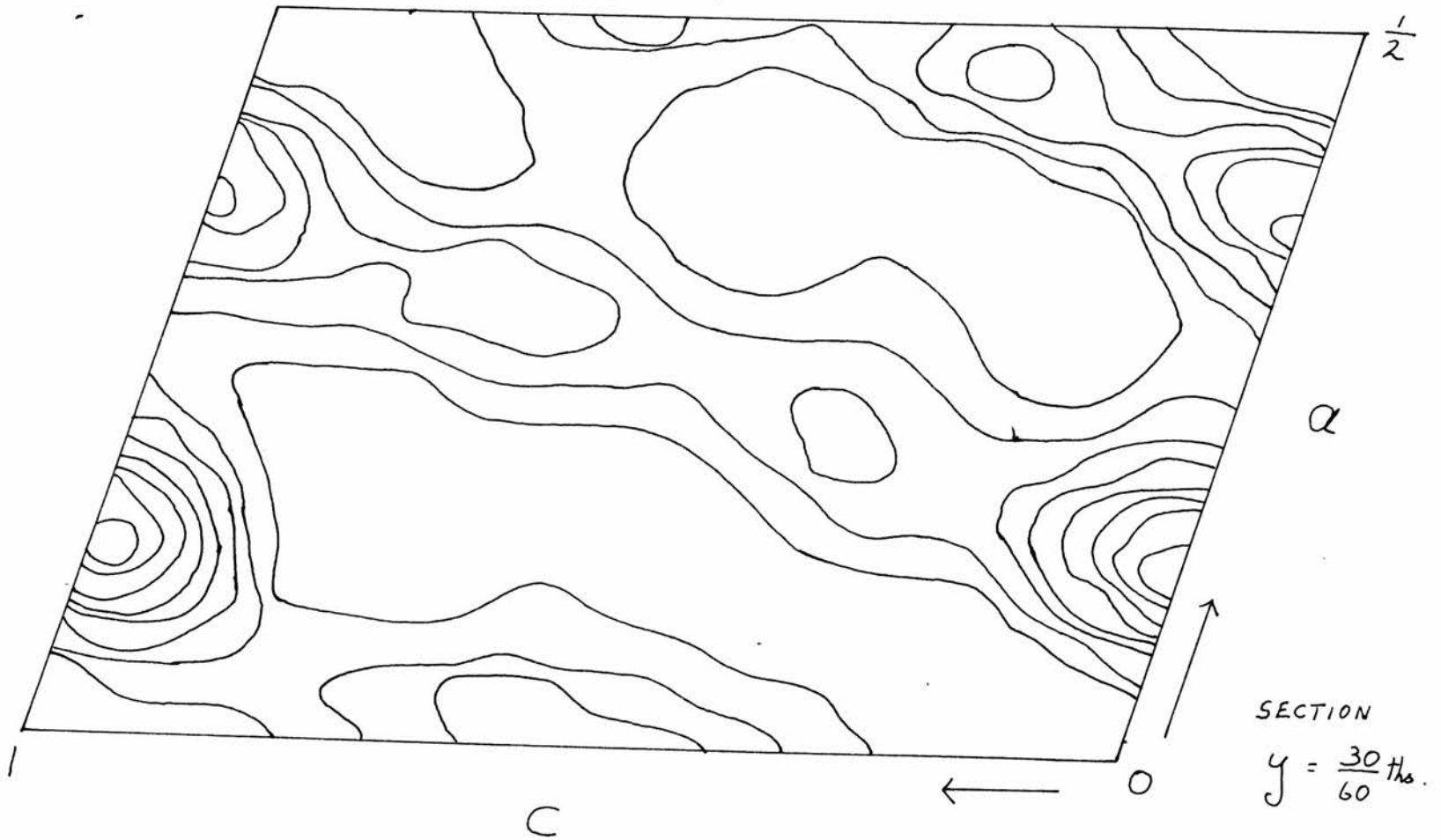


FIG. 17.

of this environment which led to the solution of the structure.

In order to facilitate identification of the intra-annular vectors around this peak, circles were drawn at a distance of 1·54Å and 2·40Å from the peak. These are shown by broken lines in the maps. In the section at $y=\frac{20}{60}$, the 2·4Å ring vector is again clearly at right-angles to the a-axis (peaks are labelled X).

The projection of vectors between atoms like C_1 and C_2, C_2 and C_3 etc. on the b-axis is about $^3/_{60}$ths. and so similar projections of vectors like C_1 and C_3, C_2 and O_3 etc. on the b-axis is about $^6/_{60}$ths. An examination of the sections separated by y = $^3/_{60}$ths. and y = $^6/_{60}$ths. from the centre of symmetry peak revealed the ring vectors quite well and confirmed the general orientation of the molecule. The ring vectors may be picked out as those interested by the broken-line circles drawn at distance 2·4Å and 1·54Å from the centre of symmetry peak. A more precise orientation of the ring was possible from the peak positions.

It was realised at this stage that another structure was possible which on the data so far obtained was indistinguishable from the previous one, and also, it would be able to take part in a hydrogen-bonding scheme similar to the one envisaged. This structure involved placing /

placing the C_1 on the glide plane, putting C_4 at same y-co-ordinate as C_1 had been at (i.e. y = 10/120ths.) then reflecting the molecule through a mirror plane parallel to the a-axis projection. This meant that C_1 had a higher x-co-ordinate than C_4 and that C_4 in turn had a higher x-co-ordinate than O_4. With this orientation, it is impossible for the centre of the molecule to be at x = 15/120ths. because this would make O_4 far too close to the centre of symmetry, so x = 45/120 was chosen as the x-co-ordinate of the centre of the molecule.

The co-ordinates of the other atoms were put in using the orientation indicated by the three-dimensional Patterson map and water molecules were included with y- and z- co-ordinates equal to those of O_3 and O_5 but with the x-co-ordinate increased by $\frac{1}{2}$. Structure-factors (h k 0) were calculated and gave as R-factor of 41·7% which refined in two cycles of Difference synthesis to 33·0%.

(d) Refinement of the Structure.

(i) Fourier Method.

The deciding information yielded by the three-dimensional Patterson was the accurate x-co-ordinates of the atoms of the molecule. Accurate z-co-ordinates could not be obtained from this Patterson but fairly accurate y- and z- co-ordinates were already to hand from the refined a-axis projection. So three-dimensional structure-factors were calculated using the y- and z- co-ordinates from the refined projection and the x-co-ordinates indicated by the three-dimensional Patterson. The resulting structure-factors S.F.1. gave an R-factor of 35·5%. A Fourier synthesis (F_o) was done with the signed $F_{obs.}(hkl)$'s and the atomic x-co-ordinates refined. The R-factor of the resulting structure-factors dropped to 29·2%. The maxima of the Fourier peaks were calculated with the aid of Booth's tables (29).

Three cycles of refinement were then carried out in which the Booth 'back-shift' was applied. Since only a Fourier series with an infinite number of terms can represent point atoms, the atoms represented by a series computed using the limited number of reflection data obtainable from the X-ray work, appear as peaks with finite width surrounded by negative and smaller, more distant positive diffraction ripples. These ripples can alter the positions of the maxima of other peaks in the Fourier synthesis /

synthesis, and Booth's 'back-shift' is an effort to allow for this effect. It involves making an Fc Fourier synthesis with certain co-ordinates $\underline{r}_n$ and also an Fo synthesis. By comparing the $\underline{r}_n$ values used with the actual positions of the peak maxima obtained in the Fc Fourier, the distance $\underline{\delta r}_n$ which the diffraction effect has moved any peak maximum from its true position may be measured. It is assumed that the Fo Fourier will suffer identical diffraction effects so in calculating the true maximum of the Fo Fourier, $\underline{\delta r}_n$ is subtracted from the maximum observed in the actual map. When this was done, the R-factor of the (h k l) reflections was reduced after three cycles to 25·0%.

An expected prominent feature of these three-dimensional Fourier maps was the streaking of the peaks parallel to the c-axis due to the lack of high order ℓ reflections, so the maps were not used to refine z-co-ordinates. It was also noted at this stage that the scaling between reflections of the ℓ layer-lines was a bit out, so this was rectified and it was decided to go on to Least Squares refinement.

(ii) Method of Least Squares.

Structure refinement is essentially a matter of varying the parameters of the atoms in the crystal until the Fc's give the best agreement with the Fo's. In following the extent of this agreement the R-factor based on $\sum(|F_o|-|F_c|)$ is normally used and an effort is made to reduce the R-factor as far as possible i.e. to minimise $\sum(|F_o|-|F_c|)$.

Now the Fo values themselves as derived from the photographs, are subject to a small range of error, and so refinement of a structure consists of the problem of getting the best fit of the Fc's with a set of Fo's which are themselves subject to error.

The principle of least-squares states that the best set of values of Fc's to be derived from the Fo's is obtained by minimising

$$R = \sum w(|Fo| - |Fc|)^2$$

where w is some weighting function. The weighting function w is useful because it may be used to vary the contribution of any one $(|Fo| - |Fc|)^2$ term to the total expression being minimised, depending on the reliability of the individual Fo measurement. If then, any Fo values are particularly open to doubt, they may be lightly weighted accordingly. Properly,

$$w(h\ k\ l) = \frac{1}{\sigma^2}(h\ k\ l)$$

where /

where σ is the probable error in $F_o(h\ k\ l)$.

In structure refinement where we are looking for δp_n^s, for any particular reflection, we can say (leaving out weights)

$$\sum_1^n \frac{\partial F}{\partial p_1} \cdot \delta p_1 = F_o - F_c = \delta F$$

where p_1, p_2 .....p_n are the n parameters determining the Fc's, and whose values must be found.

For any individual F we may write

$$\delta F_1 = F_{1o} - F_{1c}$$

$$= F_{1c} + \left[\frac{\partial F_1}{\partial p_1} \cdot \delta p_1 + \frac{\partial F_1}{\partial p_2} \cdot \delta p_2 + \frac{\partial F_1}{\partial p_3} \cdot \delta p_3 + \cdots\cdots \right] - F_{1c}$$

i.e. $$\frac{\partial F_1}{\partial p_1} \cdot \delta p_1 + \frac{\partial F_1}{\partial p_2} \cdot \delta p_2 + \frac{\partial F_1}{\partial p_3} \cdot \delta p_3 + \cdots\cdots - \delta F_1 = 0$$

and similar expressions for all the F's from F_1 to F_m.
Now, to ~~examine~~ minimise $\sum \delta F^2$, it is necessary to evaluate $\frac{\partial \sum_1^n \sum_1^m \delta F_1^2}{\partial p_1}$ and equate it to zero.
This gives

$$2 \left[\frac{\partial F_1}{\partial p_1} \cdot \delta p_1 + \frac{\partial F_1}{\partial p_2} \cdot \delta p_2 + \frac{\partial F_1}{\partial p_3} \cdot \delta p_3 + \cdots\cdots - \delta F_1 \right] \cdot \frac{\partial F_1}{\partial p_1} = 0$$

which is, writing out a few other analogous expressions,

(see following page)

$$\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_1}\right)^2\cdot\delta p_1+\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_1}\right)\left(\frac{\partial F_i}{\partial p_2}\right)\cdot\delta p_2\cdots\cdots\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_1}\right)\left(\frac{\partial F_i}{\partial p_n}\right)\cdot\delta p_n=\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_1}\right)\cdot\delta F_i$$

$$\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_2}\right)\left(\frac{\partial F_i}{\partial p_1}\right)\cdot\delta p_1+\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_2}\right)^2\cdot\delta p_2\cdots\cdots\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_2}\right)\left(\frac{\partial F_i}{\partial p_n}\right)\cdot\delta p_n=\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_2}\right)\cdot\delta F_i$$

$$\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_n}\right)\left(\frac{\partial F_i}{\partial p_1}\right)\cdot\delta p_1+\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_n}\right)\left(\frac{\partial F_i}{\partial p_2}\right)\cdot\delta p_2\cdots\cdots\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_n}\right)^2\cdot\delta p_n=\sum_{i=1}^{m}\left(\frac{\partial F_i}{\partial p_n}\right)\cdot\delta F_i$$

These are known as the normal equations, and they may be written in matrix form

$$\sum_k a_{kj}\,\epsilon_k = b_j$$

where $a_{kj}=\sum\frac{\partial F_i}{\partial p_k}\cdot\frac{\partial F_i}{\partial p_j}$, $b_j=\frac{\partial F_i}{\partial p_j}\cdot\delta F_i$, $\epsilon_k=\delta p_k$.

n is usually three times the number of atoms in the crystal though it often involves various temperature factor parameters also, and m is the number of reflections being used in /

in the refinement.

The normal equations are now to be solved for the $\sum_{j=1}^{n} \delta p_j$ values. This is possible when m > n as, of course, it invariably is in structure refinement, but it is clearly going to involve considerable labour in evaluating the co-efficients of the normal equations. A usual approximation is that of omitting all the off-diagonal terms of the matrix. As Macdonald (20) pointed out, the diagonal terms of the matrix, since they are composed of the sum of squared terms, are in general larger than the off-diagonal terms which involve the sum of both positive and negative quantities.

When this approximation is made, the expression for the parameter shifts reduces to

$$\delta p_i = \frac{\sum w \left(\frac{\partial F_i}{\partial p_i}\right) \cdot \delta F_i}{\sum w \left(\frac{\partial F_i}{\partial p_i}\right)^2}$$

The least-squares refinement on inositol-dihydrate was done on the D.E.U.C.E. Computer of the University of Glasgow using programmes devised by J.S. Rollett (30). The least-squares programme involves the above approximation and also incorporates a facility for anisotropic refinement of the temperature factor. The weighting system employed was as follows:

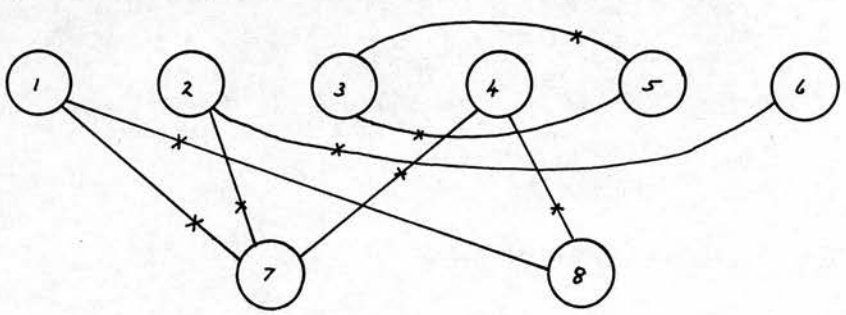
$$|F_o| \leqslant 8|F_{min.}| \quad , \quad \sqrt{w} = |F_o| / 8|F_{min.}|$$

$$|F_o| > 8|F_{min.}| \quad , \quad \sqrt{w} = 8|F_{min.}| / |F_o|$$

When the least-square refinement was started, the /

the three-dimensional R-factor was 25%, and initially no account was taken of the indications given on the anisotropic temperature factors. The original isotropic value of B = -1·19 was reinserted after each cycle of refinement and ~~unsolved~~ unobserved reflections were excluded from the refinement.

After four cycles of least-squares refinement, the R-factor was reduced to 20·8% and a three-dimensional Difference Fourier was computed. This showed up 12 of the 16 hydrogen atoms all in reasonable positions and with average electron density of about 0·6 $e/\text{Å}^2$. These hydrogens were inserted as well as the other four which were put in on bond-length and angle considerations (See P.68) Geometrically, two ways may be envisaged of allocating the hydrogen atoms among the oxygen atoms. However this anomaly was resolved by the Difference map and the hydrogen atom positions amply confirmed the postulated existence of the hydrogen-bonds between the hydroxyl groups. In fact in myo-inositol dihydrate the law of maximum hydrogen-bonding is closely obeyed. The following diagram illustrates the hydrogen positions (See page 68 for note on numbering).



After the inclusion of the hydrogen atoms in the structure-factor calculations, two further cycles of least-squares refinement were carried out - refinement being done only /

only on the carbon and oxygen atoms, and the R-factor fell to 19.7 %.

List of parameters

Atom	x/a	y/b	z/c	Atom	x/a	y/b	z/c
C_1	0.319	0.417	$\overline{0.009}$	H_1*	0.35	0.47	$\overline{0.02}$
C_2	0.262	0.362	$\overline{0.210}$	H_2	0.18	0.32	$\overline{0.21}$
C_3	0.395	0.307	$\overline{0.192}$	H_3	0.49	0.35	$\overline{0.20}$
C_4	0.450	0.256	0.011	H_4*	0.48	0.18	0.02
C_5	0.499	0.311	0.197	H_5	0.59	0.35	0.21
C_6	0.373	0.369	0.200	H_6	0.29	0.32	0.21
O_1	0.192	0.466	$\overline{0.020}$	H_7	0.27	0.00	$\overline{0.10}$
O_2	0.226	0.412	$\overline{0.406}$	H_8	0.08	0.42	$\overline{0.50}$
O_3	0.346	0.253	$\overline{0.385}$	H_9	0.20	0.25	0.50
O_4	0.323	0.207	0.015	H_{10}	0.35	0.15	0.15
O_5	0.545	0.266	0.402	H_{11}	0.48	0.25	$\overline{0.42}$
O_6	0.436	0.424	0.381	H_{12}	0.35	0.42	0.50
O_7	0.410	0.086	0.357	H_{13}	0.34	0.02	0.34
O_8	0.255	0.080	$\overline{0.321}$	H_{14}	0.35	0.08	0.50
				H_{15}*	0.25	0.11	$\overline{0.12}$
				H_{16}*	0.13	0.08	0.50

Note on numbering:

The numbering of the atoms in the molecule here is different from that on page 5. Here, the axial -OH group is on C_4. See Fig. 19.

* Hydrogen atoms not found in the three-dimensional Difference Fourier synthesis.

A few points may be made about the refinement. It appeared initially that the least-squares method should be the ideal method for making use of the substantial, yet limited, number of data possessed. But on application of the method, each cycle of refinement reduced the R-factor by only 1 - 2%. It was a slow process. One reason for this was probably the fact that the off-diagonal terms of the matrix of normal equations were neglected. As Lipson and Cochran (31) point out, if the crystal deviates far from orthogonality, then the matrix terms of the type

$$T = \sum_{i=1}^{n} \frac{\partial F_i}{\partial x_n} \cdot \frac{\partial F_i}{\partial y_n}$$ where x_n, y_n are two co-ordinates of the same atom,

become of significant magnitude and may not be neglected. In the case of myo-inositol dihydrate with $\beta \approx 120^{\circ}$ the value is too far from 90° to ignore the T type terms.

(e) Description and Discussion of Structure.

(i) Intra-molecular bonds.

The six-membered carbon ring of the inositol molecule in this structure is in the 'chair' form, and all the -OH groups are equatorial except that on C_4 which is axial. Posternak's structure (p.5) is thus fully confirmed and the question raised on this matter by the French workers (p.7) is settled as far as the crystal is concerned.

The following are the inter-atomic distances within the molecule.

$C_1 - C_2$	= 1.53Å	$C_1 - O_1$	= 1.40Å
$C_2 - C_3$	= 1.50	$C_2 - O_2$	= 1.46
$C_3 - C_4$	= 1.49	$C_3 - O_3$	= 1.47
$C_4 - C_5$	= 1.45	$C_4 - O_4$	= 1.44
$C_5 - C_6$	= 1.52	$C_5 - O_5$	= 1.45
$C_6 - C_1$	= 1.49	$C_6 - O_6$	= 1.43

The average of these distances are C - C = 1.50Å and C - O = 1.44Å.

Using least-squares formula,

$$\sigma\left(\frac{x}{a}\right) = \sum w\,(F_o - F_c)^2 \Big/ (n-s) \sum w \left[\frac{\partial(F_o - F_c)}{\partial\left(\frac{x}{a}\right)}\right]^2$$

and a programme written for D.E.U.C.E. by Dr. Sutherland of Glasgow University, values of $\sigma(x)$, $\sigma(y)$ and $\sigma(z)$ were computed for each of the carbon and oxygen atoms.

The mean values obtained were as follows

Carbon: $\sigma(x)$ = 0.012Å, $\sigma(y)$ = 0.012Å, $\sigma(z)$ = 0.018Å

Oxygen: $\sigma(x)$ = 0.008 , $\sigma(y)$ = 0.008 , $\sigma(z)$ = 0.012

Using /

Using the expression

$$\sigma^2(\ell) = [\sigma^2(x_1) + \sigma^2(x_2)] \cos^2\alpha + [\sigma^2(y_1) + \sigma^2(y_2)] \cos^2\beta + [\sigma^2(z_1) + \sigma^2(z_2)] \cos^2\gamma$$

where ℓ = bond-length

$$\cos\alpha = \frac{x_1 - x_2}{\ell}, \quad \cos\beta = \frac{y_1 - y_2}{\ell} \quad \text{and} \quad \cos\gamma = \frac{z_1 - z_2}{\ell}$$

the following results were obtained for the standard deviations of the bond-lengths.

$\sigma(C_1 - C_2) = 0.023$Å	$\sigma(C_1 - O_1) = 0.018$Å
$\sigma(C_2 - C_3) = 0.020$	$\sigma(C_2 - O_2) = 0.020$
$\sigma(C_3 - C_4) = 0.021$	$\sigma(C_3 - O_3) = 0.019$
$\sigma(C_4 - C_5) = 0.022$	$\sigma(C_4 - O_4) = 0.017$
$\sigma(C_5 - C_6) = 0.017$	$\sigma(C_5 - O_5) = 0.021$
$\sigma(C_6 - C_1) = 0.018$	$\sigma(C_6 - O_6) = 0.020$

The angles between bonds from any individual carbon atom are all quite close to the tetrahedral angle and range from 105° to 120°.

A few points may be noted about these values.

First, the abnormally short C - O bond between the axially substituted hydroxyl group and its carbon atom found by McGeachin (4) in α-rhamnose is not paralleled in inositol, so the occurrence of the short bond is probably due to the proximity of the oxygen atom in the rhamnose ring.

Secondly, it seems there is a slight but significant shortening of the C - C distance from the normal value of 1·54Å. This has also been found by Rabinowitz (32) in a study of the anhydrous crystal form of myo-inositol where /

where the average C - C distance was found to be 1·52Å. The reason for this is not clear, but it is not unique. In a recent publication, Dutta and Woolfson (34) note a number of cases in which C - C single bond distances are shorter than the accepted 1·54Å in diamond. These are listed below along with the above authors' findings.

Substance	Nature of Analysis *	C - C	Reference
L - Threonine	3 - D	1·52Å	(35)
DL - Alanine	3 - D	1·52	(36)
L - Glutamine	2 - D	1·51	(37)
Hexamethylene diamine	2 - D	1·52	(38)
∝ - Pimelic acid	3 - D	1·52	(39)
Tetraethyl diphosphine disulphide	3 - D	1·51	(34)

*3 - D = three dimensional X-ray analysis;

2 - D = two dimensional X-ray analysis.

So, as Dutta and Woolfson go on to say, it is probable that this slightly short value for the C - C single bond length is genuine. In the cases listed above explanations can be envisaged involving polarity in the molecule, but this of course would not apply to inositol.

A third point concerns the more general fact that any molecule which crystallises in a centro-symmetric space-group must itself have a centre of symmetry or at least a mirror plane. Often, however, in the case of symmetrical /

symmetrical organic molecules, it is found on refinement that they appear not to be perfectly symmetrical but seem to have significant deviations from perfect symmetry. (e.g. 33). In this case the combination of the inositol molecule and the two water molecules should have a mirror plane; but the water molecules in particular do not appear to subscribe to a symmetrical arrangement in the final structure. If this deviation is a true one which seems likely in the case of the water molecules, then the crystal could assimilate this in one of two ways. Either the centre of symmetry is not a true one and the space-group is $P2_1$ giving a pair of two independent molecules in the unit cell, or the centre of symmetry is preserved leading to two slightly enantiomorphic molecules in the crystal. The latter possibility seems the more likely in view of the evidence for the presence of a centre of symmetry in the crystals and also it does not seem unreasonable that crystal packing should involve a slight deviation from perfect symmetry.

(ii) Inter-molecular bonds.

The structure is perhaps best displayed by a view down the shortest axis - the c-axis of length 6·53Å. In this projection the cell appears with the dimensions $a \sin\beta$ = 8·49Å and b = 16·81Å. It is shown in Fig. 19. The structure is the same in its general features as that found /

found by Lomer (17), but since he only had three reflections of the type (o k l) with k = odd, it is not felt that his results for the deviation of the molecule from a symmetrical disposition about the b-axis, is as accurate as that which the above work indicates.

In the c direction perpendicular to the paper (Fig. 19) the molecules are bonded to each other through contacts $O_3 - O_5 = 2{\cdot}68\text{Å}$ and $O_2 - O_6 = 2{\cdot}77\text{Å}$, thus forming continuous chains along the c-axis. In the figure these bonds are shown as lines which are broken through where they go to atoms in molecules above or below the actual molecule shown. It is these contacts (hydrogen bonds as verified by the appearance of the hydrogen atoms in them in the three-dimensional Difference synthesis) which cause the mean plane of the molecule to be tilted some 20° ($= \beta - 90^\circ$) from the c-axis and to lie perpendicular to the a-axis. This is about the only feature of resemblance between this structure and the crystal structure of the anhydrous myo-inositol as found by Rabinowitz (32). A view down the 6·5Å axis of the latter structure reveals similar chains of inositol molecules linked together by hydrogen bonds.

The oxygen atoms 3 and 5 of the molecules in these chains are very close to the a glide planes (shown by dashed lines in Fig. 19) and bonds $O_3 - O_5$ of length 2·61Å as formed across this glide plane. These bonds not only link together the chains related to each other by the glide planes, but also hold together successive chains with the a-axis separation. This double series of molecular chains is /

is operated on either by the centres of symmetry or by the screw-axes parallel to the b-axis, to give a parallel double series which is bonded to the first by the water molecules 7 and 8.

These molecules are in pairs in the structure with an $O_7 - O_8$ distance of 2·94Å, and each water molecule makes three additional bonds; one goes to either O_2 (in the case of O_7) or O_6 (in the case of O_8) and the other two bind O_1 and O_4 of molecules of different parallel chain pairs to either O_7 or O_8, so that a tetrahedral disposition of bonds around each of the water molecules is provided. The angles between the bonds vary, however, ranging from 89° to 125° in the case of O_7 and from 89° to 114° in the case of O_8. The bond distances are quite close to the accepted O - O hydrogen-bond distances, being as follows:

$O_7 - O_1 = 2·88$Å	$O_8 - O_1 = 2·84$Å
$O_7 - O_4 = 2·90$	$O_8 - O_4 = 2·96$
$O_7 - O_2 = 2·73$	$O_8 - O_6 = 2·84$

$$O_7 - O_8 = 2·94\text{Å}$$

The average standard deviation of the hydrogen bonds (O - O) is 0·02Å.

If the $O_7 - O_8$ bond is compared with the C - C bond in ethane, it is interesting that the bonds from O_7 and O_8 to the other oxygens are conformationally 'staggered' with respect to each other, just as are the C - H bonds in ethane. The bonds to these water molecules link the structure together in all directions.

It may also be observed that the water molecules

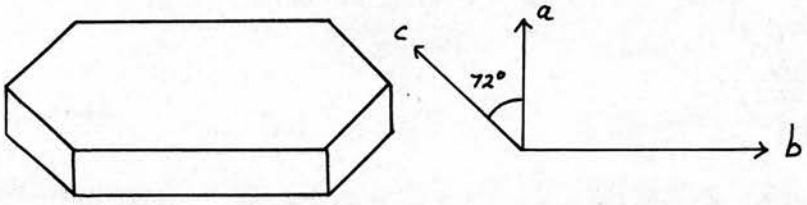
in /

in the structure all lie in sloping channels perpendicular to the b-axis and the bonds between the water molecules along these channels take up a step-wise form. It is possible that these channels provide the water molecules with easy escape routes from the crystal, and this would account for the ease with which the dihydrate crystals decompose in the atmosphere leading to a break down of the crystal structure.

In conclusion, a few general remarks can be made about the structure. The refinement has been taken far enough to confirm the correctness of the general features of the structure including the inter-molecular set-up. Further refinement, providing more accurate molecular dimensions would best be done by collecting all the three-dimensional data and using a least-squares programme capable of solving the full matrix, but it was felt that the labour which this would involve was more than would be justified by the value of the extra accuracy obtained. It would also be better to enclose the crystal in an atmosphere of high humidity because as the crystal was beginning to decompose at the end of some of the exposures, some inaccuracy would be incurred in the intensities.

3. Work on Sodium Phytate.

Sodium phytate is the duo-deca sodium salt of the hexa-phosphoric eater of myo-inositol and it is widely occuring in nature as an important phosphate constituent of the soil. The crystals used in this work were grown in water by Dr. N.J. King and were chromatographically pure. The average size of the crystals obtained was 1·5 mms. long and 0·5 mms. thick and they were well-defined in shape, having a hexagonal cross-section. Examination by optical goniometry was, therefore, an easy matter; the interfacial angles and axial ratios were measured.



The axes designated are in the direction of what later proved to be the crystallographic axes.

Oscillation and Weissenberg photographs were taken about the b - and c-axes and unit-cell dimensions obtained from high order spots on the Weissenbergs.

Values found were $a = 22{\cdot}881 \pm 0{\cdot}005\text{Å}$

$b = 12{\cdot}132 \pm 0{\cdot}005\text{Å}$

$c = 23{\cdot}066 \pm 0{\cdot}005\text{Å}$

$\beta = 108^{\circ}15' \pm 8'$

Systematic absences of reflections were noted as follows

1. Absences when $h + k$ = odd.

2. (a) Absences of $(h\ o\ l)$ when h = odd and l = odd

   Absences of $(h\ 1\ l)$ when h = even

   (b) Absences of $(o\ k\ o)$ when k = odd.

From 1, it may be deduced that the crystal is C-face centred while the conditions 2 (a) indicate a glide-plane parallel to the c-axis. Two space-groups satisfying these conditions are Cc and C2/c, the former possessing no centre of symmetry while the latter does have a centre.

The Howells, Phillips and Rogers test on the intensities was carried out and it indicated the presence of a centre. A piezo-electric test on the Giebe-Sheibe machine was negative. So it appeared that the most likely space-group was C2/c.

The density of the crystal was determined by the floatation method using bromoform and ethyl acetate and was found to be 1·72 gms./cc.

Assuming the space-group C2/c (8 molecules per unit cell) this gives a molecular weight for sodium phytate of 787 10 while the space-group Cc would give double this viz. 1574 15.

In Biochem.J. 1953,53,102 the formula for sodium phytate is given as $C_6 H_6 O_{24} P_6 Na_{12} \cdot 35H_2O$.

Two points may be made on these results.

(I) Our molecular weight would indicate 36 water molecules in the crystal - a reasonable figure considering the hexagonal nature of the molecule.

(II) If the space-group is C2/c, the molecule must be on a two-fold axis. (It cannot be on a centre of symmetry as the molecule itself is non-centrosymmetrical). Otherwise, in spite /

spite of previous evidence pointing to the presence of a centre of symmetry, the space-group is Cc. This latter possibility seems more likely.

A C K N O W L E D G M E N T S.

It is a congenial duty for the candidate to express his sincere gratitude to Dr. C.A. Beevers for the advice and inspiration which he has provided at all times and for his unflagging enthusiasm which has made this work a pleasure.

Thanks is also due to all the members of the Crystallographic Laboratory, past and present, in particular Dr. H.W. Ehrlich whose discussions and help in the early stages of this work were invaluable.

Acknowledgment is made of a maintenance grant from the D.S.I.R. during the years 1958 - 60.

REFERENCES

1. ZEIT. KRIST. 1931, 76 - 100.
2. ANGYAL, S.J. and ANDERSON, L. ADV. IN CARB.CHEM. 14, 159
3. BARTON and COOKSON, QUART. REVIEWS 10, 44.
4. McGEACHIN, H.McD. Ph.D. THESIS, UNIVERSITY OF EDINBURGH (1956).
5. PASTERNAK, R.A. ACTA CRYST. 4, 319.
6. FLECHER, ADV. IN CARB. CHEM. 3, 45.
7. ANGYAL, QUART. REVIEWS 11, 212.
8. POSTERNAK, T. HELV. CHIM. ACTA 25, 746.
9. DANGSCHAT, NATURWISS 30, 146.
10. ANGYAL and ANGYAL, J.CHEM. SOC. 1952, 695.
11. ANGYAL and McHUGH, CHEM. and IND. 1956, 1147.
12. BARRE, COURTOIS, WORMER, BULL. SOC. CHIM. BIOL. 1954, 472.
13. MAGASANIK and CHARGAFF, J.BIOL. CHEM. 1948, 173.
14. REC. TRAV. CHEM. PAYS BAS. 1948, 777.
15. BIJVOET and JELLINEK. UNPUBLISHED RESULTS.
16. BULL. SOC. CHIM. BIOL. 1954, 455 ff.
17. LOMER, PRIVATE COMMUNICATION.
18. BEEVERS, C.A. J. SCI. INST. 1952, 230.
19. HOWELLS, PHILLIPS and RODGERS. ACTA CRYST. 3, 210
20. MACDONALD T.R.R. Ph.D. THESIS, UNIVERSITY OF EDINBURGH (1950).
21. BEEVERS and EHRLICH. ZEIT. KRIST. 1959, 414.
22. COCHRAN W. ACTA CRYST. 5, 630-633.
23. BRAGG and LIPSON, ZEIT. KRIST. 95, 323
24. TAYLOR, ACTA CRYST. 7, 757.
25. TAYLOR and MORLEY, ACTA CRYST. 12, 101.
26. COCHRAN and DYER, ACTA CRYST. 5, 634.
27. DYER, ACTA CRYST. 4, 42.

28. WOOLFSON M. 'DIRECT METHODS IN CRYSTALLOGRAPHY' (1961)

29. BOOTH A.D. 'FOURIER TECHNIQUE IN X-RAY ORGANIC STRUCTURE ANALYSIS' (1948), p.64.

30. ROLLETT J.S. 'COMPUTING METHODS AND THE PHASE PROBLEM IN X-RAY CRYSTAL ANALYSIS' (1961), PAPER 7, p.87.

31. LIPSON AND COCHRAN 'THE CRYSTALLINE STATE, VOL. III' (1953) p. 282.

32. RABINOWITZ I. PRIVATE COMMUNICATION TO BE PUBLISHED.

33. EHRLICH AND BEEVERS, ACTA CRYST. 9, 605.

34. DUTTA AND WOOLFSON, ACTA CRYST. 14, 178.

35. SHOEMAKER ET AL. J.AMER. CHEM. SOC. 72, 2328.

36. DONOHUE, J.AMER. CHEM. SOC. 72, 949.

37. COCHRAN AND PENFOLD, ACTA CRYST. 5, 644.

38. BINNIE AND ROBERTSON, ACTA CRYST. 3, 424.

39. KAY AND KATZ, ACTA CRYST. 11, 289.

TABLE OF OBSERVED AND CALCULATED STRUCTURE FACTORS.

l	k	h	Fc	Fo
	2	0+	23.1-	21.4
	4	0	4.2+	4.9
	6	0	62.9-	46.4*
	8	0	8.6-	9.9
	10	0	4.9+	3.9
	12	0	17.6+	20.2
	14	0	4.3+	4.9
	18	0	25.5-	25.2
	20	0	2.5-	5.7
	1	1	10.9+	9.6
	2	1	27.7+	25.7
	3	1	24.2-	22.2
	4	1	15.2+	14.4
	5	1	10.6+	11.3
	6	1	9.7-	10.9
	7	1	9.2-	8.9
	8	1	14.4+	16.6
	10	1	7.0+	6.9
	11	1	4.7-	4.1
	12	1	1.7+	1.8
	13	1	5.8+	5.3
	15	1	2.4+	3.6
	16	1	.04+	1.8
	17	1	9.3+	8.1
	18	1	1.9+	1.8
	19	1	9.9-	8.3
	20	1	8.9-	9.1
	21	1	4.4+	3.9
	0	2	18.74-	23.4
	1	2	48.2+	38.1*
	2	2	29.9-	27.5
	3	2	3.4+	5.3
	4	2	18.8+	19.0
	5	2	7.9+	12.0
	6	2	3.5+	3.9
	7	2	16.7-	18.0
	8	2	2.3+	1.8
	9	2	15.2-	15.4
	10	2	4.1-	6.3
	11	2	6.8-	6.9
	12	2	3.2+	3.3
	13	2	.32-	1.8
	14	2	6.3+	8.5
	15	2	2.7+	1.8
	16	2	9.1+	8.5
	17	2	13.3+	14.8
	18	2	6.3+	4.7
	19	2	5.3-	5.7
	20	2	.7+	1.8
	21	2	1.3+	1.8
	1	3	14.2+	16.4
	2	3	2.6-	1.8
	3	3	1.3-	1.8
	4	3	5.9-	4.1
	5	3	18.9+	20.0
	6	3	6.1+	12.4
	7	3	17.1-	18.6
	8	3	.7-	3.3
	9	3	2.1+	1.8
	10	3	8.0-	6.9
	11	3	.7-	2.0
	13	3	3.3-	4.7
	14	3	8.6-	9.0
	15	3	3.8-	5.9
	16	3	3.7+	3.3
	17	3	2.8-	1.8
	18	3	5.0+	3.9
	19	3	.3+	1.9
	20	3	4.1+	3.9
	0	4	7.5+	4.9
	1	4	7.0-	9.1
	2	4	7.5+	4.1
	3	4	31.4+	27.5
	4	4	5.4-	4.9
	5	4	5.1+	6.3
	6	4	3.0-	1.8
	7	4	6.6+	7.7
	8	4	4.8-	3.3
	9	4	5.2-	4.7
	10	4	1.2+	1.8

l	k	h	Fc	Fo
	11	4+	1.6-	1.8
	12	4+	5.9+	4.9
	13	4+	6.6+	7.1
	14	4+	2.9+	4.7
	15	4+	0.3+	1.8
	16	4+	2.6-	1.8
	17	4+	1.0+	2.7
	18	4+	1.6-	2.7
	19	4+	2.1-	3.3
	20	4+	2.8-	2.7
	1	5+	7.3-	7.1
	2	5+	0.8+	1.8
	3	5+	1.6-	1.8
	4	5+	3.1-	3.9
	5	5+	7.5-	7.9
	6	5+	1.7-	1.8
	7	5+	1.0+	3.3
	8	5+	0.9+	1.8
	9	5+	0.8-	1.8
	10	5+	3.0-	4.1
	11	5+	5.6+	5.7
	12	5+	1.2+	1.8
	13	5+	6.4+	7.1
	14	5+	3.8-	1.8
	15	5+	1.9-	1.8
	16	5+	4.9+	3.8
	17	5+	3.2+	1.8
	18	5+	3.5-	3.3
	19	5+	1.2-	2.7
	0	6+	5.3-	4.5
	1	6+	7.5-	6.7
	2	6+	13.3-	12.7
	3	6+	1.0+	1.8
	4	6+	17.0+	16.6
	5	6+	3.4-	1.8
	6	6+	10.3+	9.9
	7	6+	0.6+	1.9
	8	6+	3.2+	2.7

l	k	h	Fc	Fo
	9	6+	6.1-	4.9
	10	6+	2.4-	3.3
	11	6+	5.4-	6.3
	12	6+	9.1-	7.9
	13	6+	2.7-	4.1
	14	6+	8.3-	7.7
	15	6+	2.7+	4.1
	16	6+	1.7+	1.8
	17	6+	1.1+	1.8
	18	6+	2.7+	2.7
	1	7+	5.4+	4.9
	2	7+	1.0+	1.8
	3	7+	1.5-	4.5
	4	7+	20.1+	18.6
	5	7+	2.0-	1.8
	6	7+	.04+	1.8
	7	7+	.5-	1.8
	8	7+	.1-	1.8
	9	7+	.9+	1.8
	10	7+	9.5-	8.1
	11	7+	3.7+	4.1
	13	7+	4.4-	3.9
	14	7+	8.6+	7.1
	15	7+	0.6-	1.8
	16	7+	.06-	1.9
	1	8+	.9+	1.9
	2	8+	1.1-	1.8
	3	8+	3.7-	3.3
	4	8+	10.2-	10.5
	5	8+	4.2+	1.8
	6	8+	8.7+	7.9
	7	8+	3.4-	3.3
	8	8+	.6+	1.8
	9	8+	3.6+	1.8
	10	8+	2.1+	1.8
	11	8+	1.1-	1.9
	12	8+	3.2-	3.3
	13	8+	3.4+	3.9
	14	8+	1.4+	3.9

l	k	h	Fc	Fo
	1	9+	3.3-	1.8
	2	9+	.2-	1.8
	3	9+	6.5-	8.1
	4	9+	5.0-	5.3
	5	9+	9.8+	7.5
	6	9+	6.0-	8.1
	7	9+	6.7+	5.7
	8	9+	.4+	1.8
	9	9+	1.6-	2.7
	10	9+	3.4+	3.9
	11	9+	1.3-	2.7
	1	10+	4.3-	5.9
	2	10+	4.6+	2.7
	3	10+	1.4-	4.5
	4	10+	3.6-	3.3
	5	10+	6.3-	7.7
	6	10+	9.7+	8.3
	7	10+	3.9+	4.5
	8	10+	8.7-	9.7
1	0	0+	2.4+	2.7
1	1	0+	.8+	1.8
1	2	0+	6.7-	5.9
1	3	0+	.5-	1.8
1	4	0+	11.8+	11.5
1	5	0+	1.4+	2.7
1	6	0+	36.0+	29.9
1	7	0+	2.3-	1.8
1	8	0+	5.2-	4.9
1	9	0+	3.1+	3.7
1	10	0+	2.2+	1.8
1	11	0+	2.3-	1.8
1	12	0+	24.4-	22.4
1	13	0+	2.8+	1.8
1	14	0+	3.5-	1.8
1	15	0+	0.1+	1.8
1	16	0+	0.3-	1.8
1	17	0+	1.7+	2.7
1	18	0+	0.06-	1.8
1	19	0+	5.0-	4.7
1	20	0+	3.2+	1.8
1	21	0+	1.9+	1.9
1	1	1+	4.1-	4.7
1	2	1+	1.1-	1.8
1	3	1+	4.5+	5.3
1	4	1+	19.7+	20.8
1	5	1+	17.4-	17.8
1	6	1+	.9+	1.8
1	7	1+	4.8+	5.3
1	8	1+	11.6+	10.5
1	9	1+	5.9-	5.7
1	10	1+	.1-	1.8
1	11	1+	9.7+	7.9
1	12	1+	4.4+	3.7
1	13	1+	.3-	1.8
1	14	1+	3.8+	4.9
1	15	1+	4.4+	4.1
1	16	1+	.6-	1.8
1	17	1+	.9+	1.8
1	18	1+	2.5-	1.8
1	19	1+	.5-	1.8
1	20	1+	3.9-	3.7
1	21	1+	3.1-	2.7
1	0	2+	7.2-	5.3
1	1	2+	21.7-	19.8
1	2	2+	17.5+	16.6
1	3	2+	34.3-	30.3
1	4	2+	27.6-	27.1
1	5	2+	21.4-	19.8
1	6	2+	8.5-	10.5
1	7	2+	8.4+	7.5
1	8	2+	.8-	1.8
1	9	2+	1.8+	1.9
1	10	2+	4.5+	3.7
1	11	2+	15.4+	12.8
1	12	2+	8.3+	6.7
1	13	2+	9.3-	9.3

l	k	h	Fo	Fo
1	14	2+	2.5+	1.8
1	15	2+	4.4-	3.7
1	16	2+	1.0+	1.8
1	17	2+	7.6-	6.9
1	18	2+	1.7-	1.8
1	19	2+	3.7+	1.9
1	20	2+	1.2-	1.9
1	1	3+	.6-	1.9
1	2	3+	.6-	1.9
1	3	3+	.3-	1.8
1	4	3+	.5-	1.8
1	5	3+	15.4+	15.8
1	6	3+	10.7-	6.7
1	7	3+	15.8+	14.0
1	8	3+	5.6-	4.7
1	9	3+	1.1-	3.8
1	10	3+	6.7+	7.9
1	11	3+	14.8-	14.4
1	12	3+	.2-	1.8
1	13	3+	12.0-	9.9
1	14	3+	3.1+	1.8
1	15	3+	4.1+	3.7
1	16	3+	2.8-	4.7
1	17	3+	2.0-	1.8
1	18	3+	1.9-	2.7
1	19	3+	4.3+	2.7
1	0	4+	.5+	1.8
1	1	4+	1.7+	1.8
1	2	4+	2.6+	3.7
1	3	4+	6.3+	10.3
1	4	4+	.8+	1.8
1	5	4+	5.1+	5.3
1	6	4+	6.3-	6.9
1	7	4+	8.5-	7.9
1	8	4+	3.9+	4.7
1	9	4+	25.7+	24.0
1	10	4+	7.9-	5.7
1	11	4+	5.5-	5.7

l	k	h	Fc	Fo
1	12	4+	7.1+	7.9
1	13	4+	2.0+	1.8
1	14	4+	1.1+	1.8
1	15	4+	1.5-	1.8
1	16	4+	3.5+	3.1
1	17	4+	.2-	1.8
1	18	4+	2.8-	1.9
1	19	4+	.04+	3.1
1	1	5+	1.1-	1.8
1	2	5+	9.0-	8.7
1	3	5+	6.3+	6.5
1	4	5+	10.5-	10.3
1	5	5+	11.8-	12.3
1	6	5+	2.2-	4.1
1	7	5+	9.2-	7.9
1	8	5+	2.4+	1.8
1	9	5+	7.3-	8.3
1	10	5+	14.6+	15.0
1	11	5+	13.7+	13.0
1	12	5+	11.3-	11.5
1	13	5+	7.4+	7.9
1	14	5+	.04-	1.8
1	15	5+	1.8+	1.8
1	16	5+	4.5-	3.1
1	17	5+	.7+	1.8
1	0	6+	16.9+	13.2
1	1	6+	6.3+	10.5
1	2	6+	11.3+	7.7
1	3	6+	11.5-	9.1
1	4	6+	3.4-	6.5
1	5	6+	14.2+	15.0
1	6	6+	8.4-	5.7
1	7	6+	18.2-	17.4
1	8	6+	18.4-	21.2
1	9	6+	4.9+	3.7
1	10	6+	12.8+	12.2
1	11	6+	9.7-	8.3
1	12	6+	4.0+	2.7

l	k	h	Fc	Fo
1	13	6+	7.9+	6.7
1	14	6+	4.0+	3.7
1	15	6+	6.4-	4.9
1	16	6+	.3+	1.8
1	17	6+	2.6-	3.1
1	1	7+	1.3+	1.8
1	2	7+	3.2-	1.8
1	3	7+	.8-	1.8
1	4	7+	10.9+	12.8
1	5	7+	3.8+	4.1
1	6	7+	3.2+	3.7
1	7	7+	2.1+	3.7
1	8	7+	.3-	1.8
1	9	7+	3.7-	1.8
1	10	7+	1.1-	1.8
1	11	7+	.4+	1.8
1	12	7+	4.1-	3.7
1	13	7+	4.5-	5.9
1	14	7+	9.5+	9.5
1	15	7+	4.0+	4.1
1	0	8+	9.2-	5.7
1	1	8+	5.5+	9.3
1	2	8+	1.3-	1.8
1	3	8+	3.4+	1.8
1	4	8+	6.2-	7.1
1	5	8+	7.6+	6.3
1	6	8+	10.2+	11.7
1	7	8+	3.9-	3.1
1	8	8+	1.5-	1.8
1	9	8+	4.3-	2.7
1	10	8+	.9+	1.8
1	11	8+	4.3-	3.7
1	12	8+	5.1-	7.5
1	13	8+	.4-	1.8
1	1	9+	7.9+	7.3
1	2	9+	.4-	3.1
1	3	9+	3.1-	4.1
1	4	9+	3.1-	2.7
1	5	9+	1.1-	3.1
1	6	9+	.3+	1.8
1	7	9+	7.5-	5.7
1	8	9+	2.9-	3.1
1	9	9+	1.1+	1.8
1	10	9+	1.0+	3.1
1	0	10+	4.3+	2.7
1	1	10+	1.1-	3.7
1	1	1-	3.3+	10.9
1	2	1-	22.2-	16.4
1	3	1-	16.0-	11.5
1	4	1-	16.2-	12.3
1	5	1-	3.1-	4.9
1	6	1-	2.6-	2.7
1	7	1-	2.6-	2.7
1	8	1-	12.5-	12.7
1	9	1-	1.3+	1.8
1	10	1-	5.8-	4.7
1	11	1-	8.0+	7.9
1	12	1-	5.0+	4.7
1	13	1-	4.2-	4.7
1	14	1-	2.2-	1.8
1	15	1-	.7-	4.1
1	16	1-	2.6+	1.8
1	17	1-	3.7-	4.7
1	18	1-	8.0-	7.3
1	19	1-	.07-	3.7
1	20	1-	6.3+	5.9
1	21	1-	6.7+	4.7
1	0	2-	28.2-	20.4
1	1	2-	45.5-	33.3 *
1	2	2-	26.5-	27.3
1	3	2-	4.3-	4.7
1	4	2-	17.7+	14.0
1	5	2-	4.5-	4.7
1	6	2-	1.3+	1.8
1	7	2-	14.4+	13.2
1	8	2-	7.8+	6.5

l	k	h	Fc	Fo
1	9	2-	16.5+	16.4
1	10	2-	4.2-	4.9
1	11	2-	2.7+	1.8
1	12	2-	8.1+	7.7
1	13	2-	2.3+	3.1
1	14	2-	10.4-	9.1
1	15	2-	7.6-	6.9
1	16	2-	8.5+	7.5
1	17	2-	8.1-	6.9
1	18	2-	.1+	1.8
1	19	2-	3.3+	3.1
1	20	2-	7.5+	5.3
1	21	2-	4.0+	1.9
1	1	3-	.2-	1.8
1	2	3-	8.7-	6.9
1	3	3-	.4+	1.8
1	4	3-	22.2+	16.4
1	5	3-	31.6+	33.3
1	6	3-	11.2-	14.0
1	7	3-	14.3-	14.4
1	8	3-	17.1+	20.0
1	9	3-	.9+	2.7
1	10	3-	1.4-	3.1
1	11	3-	.8+	1.8
1	12	3-	1.2-	1.8
1	13	3-	9.2-	7.5
1	14	3-	4.0-	1.8
1	15	3-	4.1-	3.7
1	16	3-	4.6+	4.9
1	17	3-	1.4-	1.8
1	18	3-	1.9-	1.8
1	19	3-	3.2+	4.9
1	20	3-	4.7+	3.7
1	0	4-	13.2-	8.9
1	1	4-	1.4+	1.8
1	2	4-	12.0-	14.4
1	4	4-	12.8+	11.7
1	5	4-	14.6-	16.6
1	6	4-	12.6+	11.5
1	7	4-	11.7-	13.8
1	8	4-	7.2+	6.5
1	9	4-	31.8-	32.1
1	10	4-	3.6-	1.8
1	11	4-	1.9+	1.8
1	12	4-	5.1+	3.7
1	13	4-	10.1-	10.9
1	14	4-	7.3-	6.9
1	15	4-	23.4+	20.2
1	16	4-	1.9+	1.8
1	17	4-	2.8+	1.8
1	18	4-	2.8+	1.8
1	20	4-	2.6+	1.9
1	1	5-	4.2-	5.7
1	2	5-	4.2	3.7
1	3	5-	8.2-	6.7
1	4	5-	16.3+	18.2
1	5	5-	11.3-	16.2
1	6	5-	15.0-	15.4
1	7	5-	9.4+	9.9
1	8	5-	10.6+	12.2
1	9	5-	2.6+	1.8
1	10	5-	.8-	1.8
1	11	5-	.3-	1.8
1	12	5-	2.7-	1.8
1	13	5-	4.7+	1.8
1	14	5-	2.3-	1.8
1	15	5-	1.0+	1.8
1	16	5-	3.1+	6.5
1	17	5-	2.4+	5.7
1	18	5-	1.7-	3.7
1	19	5-	2.1-	2.7
1	0	6-	19.5+	22.0
1	1	6-	16.4-	23.4
1	2	6-	8.9+	8.7
1	3	6-	9.6+	10.3
1	4	6-	9.5-	13.1

l	k	h	Fc	Fo
1	5	6-	2.0-	1.8
1	6	6-	5.7-	6.5
1	7	6-	6.9+	8.7
1	8	6-	6.7-	7.7
1	9	6-	4.3+	5.9
1	10	6-	6.1+	7.5
1	11	6-	1.1+	1.8
1	12	6-	2.3-	4.9
1	13	6-	1.7-	4.9
1	14	6-	8.2+	8.5
1	15	6-	.1+	1.8
1	16	6-	5.6-	5.7
1	17	6-	3.5-	3.7
1	18	6-	3.2-	2.7
1	1	7-	.3-	1.8
1	2	7-	.3-	1.8
1	3	7-	4.0+	4.1
1	4	7-	.1+	1.8
1	5	7-	3.9+	4.9
1	6	7-	1.9+	1.8
1	7	7-	1.4+	1.8
1	8	7-	6.3-	5.9
1	9	7-	.7-	1.8
1	10	7-	4.1-	4.9
1	11	7-	6.1-	5.3
1	12	7-	1.5-	3.7
1	13	7-	.2-	1.8
1	14	7-	.6-	1.8
1	15	7-	.9-	1.8
1	16	7-	4.7+	3.1
1	17	7-	3.0+	1.9
1	1	8-	1.2+	1.8
1	2	8-	1.0+	1.8
1	3	8-	5.7+	5.7
1	4	8-	1.5-	1.8
1	5	8-	2.9+	3.7
1	6	8-	5.6-	4.7
1	7	8-	3.6-	3.7
1	8	8-	3.6+	4.1
1	9	8-	3.4-	3.1
1	10	8-	.6-	2.7
1	11	8-	5.1-	4.7
1	12	8-	4.9+	4.9
1	13	8-	1.7+	1.8
1	15	8-	3.5+	2.7
1	1	9-	1.9+	4.1
1	2	9-	1.1+	3.7
1	3	9-	3.9-	4.7
1	4	9-	5.2+	4.9
1	5	9-	4.0+	1.8
1	6	9-	1.6+	1.8
1	7	9-	3.6-	1.8
1	8	9-	1.8+	1.8
1	0	10-	8.8+	6.7
1	1	10-	3.9-	3.1
1	2	10-	.9+	4.7
1	3	10-	5.4-	5.3
1	4	10-	2.7+	3.1
1	5	10-	1.3-	2.7
1	6	10-	2.8-	3.1
1	7	10-	3.3+	4.7
1	8	10-	5.7-	2.7
2	1	0+	3.3-	1.8
2	2	0+	1.7+	1.8
2	3	0+	6.8+	7.5
2	4	0+	29.9+	24.4
2	5	0+	4.2+	4.7
2	6	0+	16.3-	21.0
2	7	0+	5.6+	5.3
2	8	0+	2.5+	3.3
2	9	0+	4.0-	4.3
2	10	0+	1.7-	1.8
2	11	0+	1.2+	1.8
2	12	0+	1.5+	2.7
2	13	0+	2.9-	1.8
2	14	0+	5.9-	7.9

l	k	h	Fo	Fo
2	15	0+	5.4+	4.3
2	16	0+	8.4-	9.7
2	17	0+	2.5-	1.8
2	18	0+	.3+	1.8
2	19	0+	2.9+	1.8
2	20	0+	2.8+	3.9
2	1	1+	7.1-	8.5
2	2	1+	2.8+	3.9
2	3	1+	7.6+	9.2
2	4	1+	31.0+	29.7
2	5	1+	18.4-	15.6
2	6	1+	13.4+	109
2	7	1+	2.3-	1.9
2	8	1+	7.6-	6.7
2	9	1+	9.4+	9.1
2	10	1+	8.9-	8.5
2	11	1+	0.06+	1.8
2	12	1+	3.3-	1.8
2	13	1+	12.8+	11.8
2	14	1+	14.7+	13.4
2	15	1+	11.6-	107
2	16	1+	3.3-	5.3
2	17	1+	4.2+	3.9
2	18	1+	2.1+	1.8
2	0	2+	8.3-	7.9
2	1	2+	19.7+	16.2
2	2	2+	24.2-	20.8
2	3	2+	3.1+	1.8
2	4	2+	14.0+	13.8
2	5	2+	24.6+	23.4
2	6	2+	4.1-	2.7
2	7	2+	22.1-	18.0
2	8	2+	31.3+	33.0
2	9	2+	17.5-	17.4
2	11	2+	13.6-	17.8
2	12	2+	1.5+	1.8
2	13	2+	6.5+	6.9
2	14	2+	3.3-	3.3
2	15	2+	9.7+	9.3

l	k	h	Fc	Fo
2	16	2+	8.3+	7.2
2	17	2+	2.3+	1.8
2	18	2+	2.3+	2.7
2	1	3+	9.2+	6.3
2	2	3+	5.9-	3.9
2	3	3+	3.4-	3.3
2	4	3+	1.6-	3.9
2	5	3+	6.8+	5.9
2	6	3+	5.0-	3.9
2	7	3+	1.4-	2.7
2	8	3+	1.9+	3.3
2	9	3+	9.3+	8.3
2	10	3+	9.4+	8.5
2	11	3+	15.1-	15.4
2	12	3+	10.4-	9.7
2	13	3+	2.0+	1.8
2	14	3+	3.2+	3.3
2	15	3+	3.1-	3.9
2	16	3+	4.4-	6.9
2	0	4+	12.7-	11.3
2	1	4+	5.6-	4.7
2	2	4+	6.2-	5.9
2	3	4+	11.25-	8.3
2	4	4+	4.2+	12.0
2	5	4+	1.6+	2.7
2	6	4+	4.3+	4.7
2	7	4+	3.0-	3.9
2	8	4+	10.4+	9.1
2	9	4+	31.5+	28.1
2	10	4+	1.9-	2.0
2	11	4+	2.9-	4.3
2	12	4+	4.3-	4.3
2	13	4+	.3+	1.8
2	14	4+	4.4-	2.7
2	16	4+	.8-	1.8
2	17	4+	1.8-	1.8
2	1	5+	8.7-	6.9
2	2	5+	6.9+	7.5

l	k	h	Fc	Fo
2	3	5+	2.1-	1.8
2	4	5+	6.3+	5.2
2	5	5+	.6-	1.8
2	6	5+	4.0-	5.7
2	7	5+	1.5-	1.8
2	8	5+	10.7-	8.3
2	9	5+	4.8+	3.0
2	10	5+	2.7+	1.8
2	11	5+	1.1+	1.8
2	12	5+	1.5-	1.8
2	13	5+	2.5+	1.8
2	14	5+	4.4+	4.7
2	15	5+	3.4-	3.3
2	16	5+	3.3+	2.7
2	0	6+	10.9+	1.8
2	1	6+	5.4-	9.3
2	2	6+	2.8-	1.8
2	3	6+	.5+	1.8
2	4	6+	5.3+	5.0
2	5	6+	5.0-	5.7
2	6	6+	.4-	1.8
2	7	6+	.8-	1.8
2	8	6+	2.5-	2.7
2	9	6+	2.1-	1.8
2	10	6+	1.2-	1.8
2	11	6+	1.3+	1.8
2	12	6+	4.2-	4.7
2	13	6+	2.3-	1.8
2	14	6+	2.2+	2.7
2	1	7+	2.2-	1.8
2	2	7+	2.7-	4.7
2	3	7+	1.1+	1.8
2	4	7+	.8+	1.8
2	5	7+	5.0-	4.7
2	6	7+	3.5-	4.9
2	7	7+	.9+	1.8
2	8	7+	9.2+	7.5
2	9	7+	.2+	1.8

l	k	h	Fc	Fo
2	10	7+	4.2	3.9
2	11	7+	4.0-	3.9
2	12	7+	2.7+	1.9
2	0	8+	4.6-	1.8
2	1	8+	.3+	1.8
2	2	8+	2.6-	4.5
2.	3	8+	4.9-	4.5
2	4	8+	2.6+	5.7
2	5	8+	4.3-	2.7
2	6	8+	6.3-	4.5
2	7	8+	2.8+	4.9
2	8	8+	3.1+	3.3
2	9	8+	1.7+	2.7
2	1	9+	3.7-	1.8
2	2	9+	4.7-	4.9
2	3	9+	3.5+	4.3
2	4	9+	3.3-	3.9
2	5	9+	3.1+	1.9
2	1	1-	5.8-	6.0
2	2	1-	6.3-	5.9
2	4	1-	32.3	35.5
2	5	1-	5.1-	7.5
2	6	1-	3.4+	3.3
2	7	1-	3.7-	4.3
2	8	1-	3.4	-3.9
2	9	1-	4.5-	4.7
2	10	1-	9.6+	7.5
2	11	1-	1.2-	3.9
2	12	1-	10.2-	7.9
2	13	1-	8.9+	9.1
2	14	1-	9.4	6.9
2	15	1-	.1-	2.7
2	16	1-	2.0+	1.8
2	17	1-	.8+	2.7
2	18	1-	3.2+	1.8
2	19	1-	.6-	1.8
2	20	1-	4.2+	3.9
2	0	2-	11.7-	6.0

l	k	h	Fc	Fo
2	1	2-	26.2+	18.6
2	2	2-	20.3+	17.4
2	3	2-	23.0+	23.4
2	4	2-	25.3-	23.4
2	5	2-	18.0+	17.2
2	6	2-	11.4-	12.9
2	7	2-	12.0-	13.6
2	8	2-	5.5-	5.9
2	9	2-	5.0-	5.3
2	10	2-	3.7+	1.8
2	11	2-	8.6-	6.7
2	12	2-	10.8+	9.1
2	13	2-	5.3+	3.9
2	14	2-	9.4+	9.1
2	15	2-	5.5+	1.8
2	16	2-	.1	1.8
2	17	2-	4.1+	1.8
2	18	2-	1.3+	1.8
2	1	3-	4.2+	7.5
2	2	3-	8.0+	7.5
2	3	3-	.3+	1.8
2	4	3-	11.9+	13.0
2	5	3-	13.7+	12.8
2	6	3-	4.4+	4.7
2	7	3-	.7-	1.8
2	8	3-	2.0-	3.3
2	9	3-	1.3+	1.8
2	10	3-	3.3+	3.9
2	11	3-	4.4-	5.3
2	12	3-	2.2-	1.8
2	13	3-	9.0-	7.9
2	14	3-	5.1+	4.7
2	15	3-	3.1+	1.8
2	16	3-	2.2-	1.8
2	17	3-	3.7-	1.8
2	19	3-	4.2+	2.7
2	0	4-	10.2+	9.7
2	1	4-	10.0+	7.9

l	k	h	Fc	Fo
2	1	4-	10.0+	7.9
2	3	4-	28.7-	27.0
2	4	4-	.7-	1.8
2	5	4-	6.5-	6.7
2	6	4-	8.2-	7.1
2	7	4-	6.5-	5.9
2	8	4-	2.6-	1.8
2	9	4-	7.4+	8.3
2	10	4-	0	1.8
2	11	4-	1.8+	1.8
2	12	4-	3.3+	3.9
2	13	4-	7.8-	7.1
2	14	4-	1.2-	1.8
2	15	4-	2.0-	1.8
2	16	4-	2.1+	4.7
2	17	4-	.5-	1.8
2	18	4-	3.6-	1.9
2	19	4-	3.9+	2.7
2	1	5-	2.4-	1.8
2	2	5-	2.5+	3.3
2	3	5-	.6+	1.8
2	4	5-	11.2+	11.7
2	5	5-	15.6-	19.4
2	6	5-	10.9-	9.3
2	7	5-	4.1+	4.3
2	8	5-	1.1+	1.8
2	10	5-	2.3+	1.8
2	13	5-	5.3+	4.7
2	14	5-	5.7+	4.7
2	15	5-	8.7+	6.9
2	0	6-	19.6+	20.4
2	1	6-	14.7-	13.6
2	3	6-	7.1+	9.1
2	4	6-	11.7-	10.1
2	5	6-	.6-	1.8
2	6	6-	6.7-	5.7
2	7	6-	7.2+	7.3
2	8	6-	4.3-	2.7

l	k	h	Fc	Fo
2	9	6-	4.3+	4.9
2	10	6-	2.5-	1.8
2	11	6-	3.0+	1.8
2	12	6-	.06+	1.8
2	13	6-	.5+	1.8
2	14	6-	7.2+	7.1
2	15	6-	3.5+	4.5
2	16	6-	8.2-	6.3
2	1	7-	3.1+	5.9
2	2	7-	12.3-	11.1
2	3	7-	7.3+	6.3
2	4	7-	3.3-	6.7
2	5	7-	7.0-	7.7
2	6	7-	1.2+	1.8
2	7	7-	3.6+	4.9
2	8	7-	2.9-	1.8
2	9	7-	2.3-	1.8
2	10	7-	.8-	1.8
2	11	7-	.3+	1.8
2	12	7-	1.7+	1.8
2	13	7-	1.9-	1.8
2	14	7-	.3+	1.8
2	15	7-	1.3-	1.8
2	16	7-	1.2-	3.9
2	0	8-	35.6-	37.3
2	1	3-	14.1-	9.5
2	2	8-	8.5+	9.5
2	3	8-	7.9-	8.3
2	4	8-	3.1-	4.7
2	5	8-	7.6-	5.7
2	6	8-	12.8+	14.0
2	7	8-	4.3+	3.3
2	8	8-	1.4-	2.7
2	9	8-	6.8+	7.5
2	10	8-	.1-	1 .8
2	11	8-	3.7+	3.9
2	12	8-	4.8-	1.8
2	1	9-	3.0-	2.5
2	2	9-	2.1+	1.8
2	3	9-	4.9+	7.5
2	4	9-	3.7+	3.9
2	5	9-	2.4-	4.9
2	6	9-	4.1-	3.9
2	7	9-	2.0+	1.8
2	8	9-	4.6+	4.3
2	9	9-	1.9-	3.3
2	10	9-	2.8+	4.3
2	11	9-	.7+	3.3
2	0	10-	4.1-	8.3
2	1	10-	6.3+	4.7
2	2	10-	6.9+	4.7
2	3	10-	3.1#	3.9
2	4	10-	2.5-	3.3
2	5	10-	1.2+	2.7
2	6	10-	3.3+	5.7
2	7	10-	5.2-	1.8
2	8	10-	.07+	2.7
2	9	10-	3.0-	3.3
2	10	10-	.8+	1.7
3	0	0+	37.5+	35.9
3	1	0+	6.2-	4.7
3	2	0+	9.8+	7.5
3	3	0+	.8+	2.9
3	4	0+	12.6+	12.0
3	5	0+	1.0-	1.8
3	6	0+	34.8-	26.4
3	7	0+	.7-	1.8
3	8	0+	5.0-	5.5
3	9	0+	3.6+	4.3
3	10	0+	.7-	1.8
3	11	0+	2.7+	1.8
3	12	0+	15.7+	10.7
3	13	0+	4.2+	5.5
3	14	0+	1.5+	1.8
3	15	0+	3.7-	4.7
3	16	0+	7.4-	6.5

l	k	h	Fc	Fo
3	17	0+	.9-	1.8
3	18	0+	6.5-	3.7
3	1	1+	15.1-	13.1
3	2	1+	11.5-	13.1
3	3	1+	6.8+	5.9
3	4	1+	6.9+	5.5
3	5	1+	1.9	1.8
3	6	1+	3.7-	1.8
3	7	1+	8.0+	6.3
3	8	1+	11.9+	10.7
3	9	1+	4.7-	5.3
3	10	1+	1.3+	1.8
3	11	1+	6.6-	5.3
3	12	1+	5.0+	4.7
3	13	1+	6.4+	4.7
3	14	1+	.1+	1.8
3	15	1+	1.3+	1.8
3	16	1+	4.8-	4.7
3	17	1+	6.7+	4.3
3	0	2+	7.5-	5.5
3	1	2+	.5+	1.8
3	2	2+	7.4+	5.5
3	3	2+	1.0-	2.9
3	4	2+	8.7-	7.5
3	5	2+	3.5-	4.7
3	6	2+	.5-	1.8
3	7	2+	1.7-	1.8
3	8	2+	1.2-	1.8
3	9	2+	6.6-	5.3
3	10	2+	.7+	1.8
3	11	2+	3.6-	2.9
3	12	2+	2.7+	4.3
3	13	2+	2.4-	1.8
3	14	2+	3.3+	1.8
3	15	2+	2.8+	1.8
3	17	2+	2.1+	2.3
3	1	3+	11.3+	8.7
3	2	3+	2.3-	8.3
3	3	3+	3.8+	1.8
3	4	3+	3.2+	2.9

l	k	h	Fc	Fo
3	5	3+	.9+	1.8
3	6	3+	.9-	1.8
3	7	3+	9.1-	7.5
3	8	3+	4.0+	4.7
3	9	3+	1.0-	1.8
3	10	3+	5.5-	4.7
3	11	3+	2.6+	1.8
3	12	3+	.4+	1.8
3	13	3+	2.9-	1.8
3	14	3+	1.0-	1.8
3	15	3+	1.4+	2.3
3	0	4+	1.8+	1.8
31	1	4+	5.7-	3.7
3	2	4+	4.7+	5.3
3	3	4+	14.9+	12.9
3	4	4+	4.4-	3.7
3	5	4+	3.9+	1.8
3	6	4+	4.5-	4.7
3	7	4+	5.8+	5.5
3	8	4+	3.7-	1.8
3	9	4+	7.4-	6.5
3	10	4+	.3+	1.8
3	11	4+	.4+	1.8
3	12	4+	3.9+	3.7
3	13	4+	5.9 +	5.3
3	14	4+	2.0+	2.9
3	1	5+	1.9+	1.8
3	2	5+	4.5-	5.0
3	3	5+	3.7-	6.3
3	4	5+	9.5-	7.5
3	5	5+	8.4-	7.5
3	6	5+	5.1+	6.5
3	7	5+	3.6+	4.5
3	8	5+	.3-	1.8
3	1	6+	12.6+	9.5
3	6	6+	3.3+	1.8
3	7	6+	3.8+	2.3
3	8	6+	3.9+	3.7
3	9	6+	2.6-	2.9

l	k	h	Fc	Fo
3	1	7+	.5+	2.3
3	2	7+	15.1+	10.1
3	3	7+	5.5+	4.3
3	6	7+	1.0+	3.7
3	7	7+	.1+	1.8
3	1	1-	.5+	3.7
3	2	1-	6.2+	12.4
3	3	1-	13.7+	15.4
3	4	1-	13.2-	11.7
3	5	1-	11.9-	11.3
3	6	1-	8.3-	6.3
3	7	1-	8.4-	6.3
3	8	1-	4.1-	4.7
3	9	1-	4.3+	4.3
3	10	1-	3.5-	1.8
3	11	1-	1.1-	1.8
3	12	1-	3.5+	1.8
3	13	1-	12.1+	8.5
3	14	1-	9.3-	6.5
3	15	1-	9.3-	7.1
3	16	1-	7.6+	5.9
3	18	1-	2.2-	3.7
3	0	2-	20.9-	17.8
3	1	2-	15.9-	22.2
3	2	2-	13.3-	21.8
3	3	2-	7.6-	4.7
3	4	2-	5.6+	7.1
3	5	2-	30.2-	26.2
3	6	2-	2.2-	2.3
3	7	2-	23.2+	17.4
3	8	2-	24.8+	21.0
3	9	2-	12.3+	10.5
3	10	2-	17.2-	15.8
3	11	2-	15.5+	11.7
3	13	2-	12.5-	10.7
3	15	2-	7.4-	7.5
3	16	2-	5.8+	7.1
3	18	2-	5.3+	2.3
3	1	3-	1.1+	1.8
3	2	3-	1.1+	1.8
3	3	3-	6.0-	3.7
3	4	3-	1.1+	1.8
3	5	3-	8.9+	11.7
3	6	3-	.1-	1.8
3	7	3-	9.6+	5.9
3	8	3-	5.7+	5.5
3	9	3-	7.8+	7.1
3	10	3-	1.3-	1.8
3	11	3-	19.4-	19.2
3	12	3-	13.5+	11.1
3	13	3-	2.9-	1.8
3	14	3-	3.0-	3.7
3	15	3-	.1+	1.8
3	16	3-	.1+	1.8
3	18	3-	4.6-	2.3
3	1	4-	3.1+	2.3
3.	0	4-	2.3+	1.8
3	2	4-	1.4+	2.3
3	3	4-	10.4-	5.3
3	4	4-	.9+	2.3
3	5	4-	3.0-	5.3
3	6	4-	.1-	1.8
3	7	4-	4.8+	6.3
3	8	4-	4.4+	4.3
3	9	4-	20.4-	20.8
3	10	4-	.1-	1.8
3	11	4-	8.3+	7.5
3	12	4-	5.8-	5.5
3	13	4-	.8-	1.8
3	14	4-	1.6+	1.8
3	15	4-	2.5+	3.7
3	1	5-	1.9-	4.3
3	5	5-	10.45-	8.5
3	6	5-	3.5+	4.3
3	7	5-	3.3-	5.3
3	9	5-	5.3+	4.7
3	10	5-	7.1-	7.5
3	11	5-	7.4+	7.5
3	13	5-	6.4+	5.5
3	15	5-	5.0-	3.7
3	1	6-	5.2+	5.5
3	2	6-	13.2-	13.1

l	k	h	Fc	Fo
3	4	6-	13.5+	16.2
3	5	6-	6.5+	4.3
3	6	6-	6.9+	7.5
3	7	6-	2.7+	2.3
3	8	6-	3.9+	3.7
3	9	6-	4 .5+	5.5
3	12	6-	8.6-	7.5
3	13	6-	4.0+	5.5
3	4	7-	9.4-	8.7
3	5	7-	4.7+	7.5
3	10	7-	3.2+	3.7
3	11	7-	4.4-	5.9
3	0	8-	5.7-	3.7
3	2	8-	1.5-	7.5
3	3	8-	4.2+	6.5
3	6	8-	4.9-	6.5
3	7	8-	2.8-	5.5
3	8	8-	3.0+	4.7
3	9	8-	5.2-	5.5
3	11	8-	2.6-	6.5
3	12	8-	6.3+	7.5
3	01	9-	6.6-	4.7

* AFFECTED BY EXTINCTION

FIG. 19.